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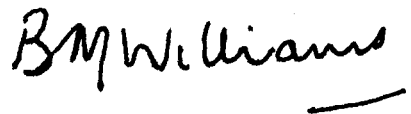
Studies on the epizootiology of
canine cestodes particularly Taenia
multiceps and ovine coenurosis

By

Bernard Milton Williams MRCS DVSM

DECLARATION

I hereby declare that this thesis has been composed by myself, that it has not been submitted previously for a higher degree, that the work of which it is a record has been done by myself or, where jointly with other workers, is accompanied by a signed declaration by these workers, and that all quotations are distinguished by quotation marks and the sources of information specifically acknowledged.

A handwritten signature in black ink, reading "BM Williams". The signature is written in a cursive style with a horizontal line underneath the name.

Bernard Milton Williams

CERTIFICATE

We certify that Bernard Milton Williams, a candidate for the Degree of Doctor of Philosophy in the Open University, has fulfilled the Regulations of the Open University for the Degree of Doctor of Philosophy by Thesis, and is qualified thereby to submit this thesis.

Dr E A Bowers

B.L. James
Dr B L James

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ABSTRACT

In a survey of 552 sheep flocks in Dyfed, Wales, carried out in 1973, coenurosis occurred in 375 (68%) with an estimated average annual flock incidence of between 2.5 and 3.0%. Evidence of infection with the larval stage of Taenia multiceps was found in 58/1000 (5.8%) lambs sent for slaughter. Taenia multiceps was recovered from 37/320 (11.5%) dogs on 20/100 sheep breeding farms, 3/39 (7.7%) dogs on 2/18 sheep wintering farms and 41/552 (7.4%) hounds in 4/12 packs. The frequency of cestode infection in farm dogs and hounds was closely associated with the feeding of uncooked meat and offal and the absence of regular anthelmintic treatment. Only one (0.6%) of 166 pet dogs and one (0.26%) of 387 foxes examined harboured Taenia multiceps and neither were considered to be of importance in the epizootiology of Taenia multiceps.

Dogs were experimentally infected with Taenia multiceps and remained infected for up to periods of six months or longer, and up to 21 proglottids were shed each day. Rabbits and mice could not be experimentally infected with oncospheres of Taenia multiceps, but lambs which were experimentally infected with the oncospheres developed acute or chronic coenurosis.

Suspensions of oncospheres in distilled water remained viable at 3 - 11°C for periods up to eight weeks, but the coenurus was viable for no longer than four days in the intact skull at 9 - 11°C.

The pathogenesis of acute and chronic coenurosis, with particular reference to the clinical signs, clinical pathology, pathology and immunology, was studied in experimentally infected lambs and selected field cases of the disease.

1.1 Introduction

Although coenurosis has been reported from all the sheep-raising countries of the world, there are considerable variations in prevalence and economic importance. It is difficult to fully understand why these variations occur, because the disease has attracted only a limited amount of attention from veterinary research workers in countries other than the USSR and more recently India and Great Britain. Consequently little is known of the epizootiology. It is evident however that the prevalence of the disease must be dependent not only on the abundance and range of the definitive carnivorous hosts and herbivorous intermediate hosts, but also on other factors.

Although coenurosis is regarded as an important nervous disease of sheep in Great Britain, there is no precise information available regarding prevalence or economic loss. The Ministry's (Ministry of Agriculture Fisheries and Food) Veterinary Investigation Centres in England and Wales and those of the Scottish Agricultural Colleges, provide a diagnostic service for farmers through their practising veterinary surgeons. The diagnoses reached by the centres are published annually as a tabulated summary by the Epidemiology Department of the Ministry's Central

Veterinary Laboratory, Weybridge, Surrey as the Veterinary Diagnosis Analysis II. For the period 1 January 1975 - 31 December 1988 a total of 816 incidents of coenurosis were recorded compared with 858 incidents of louping ill, the commonest, and regarded as the most important nervous disease of sheep in Great Britain.

It must be emphasised however that specimens received by the Veterinary Investigation Centres, represent a biased sample of animal disease in the field and the results of the laboratory examinations must therefore be interpreted with caution. In general such factors as the practising veterinary surgeons need or desire for laboratory confirmation of a clinical diagnosis, the scale of fees or charges raised by the centres and payable by farmers, and the ability of farmers to transport specimens, sometimes over considerable distances, govern the type and number of specimens submitted to the centres. Coenurosis, especially the chronic form, is a well recognised disease of sheep by both practising veterinary surgeons and farmers. Only a small proportion of incidents therefore are referred to Centres, for example when the disease first appears in a flock, or when the acute form involving a number of animals simultaneously occurs.

Some information has been obtained on the epizootiology of the disease in this country through studies of hydatidosis, but with no specific information, apart from recent work at University College of North Wales, Bangor which is referred to in the following sections. In spite of this it is considered by many that coenurosis is responsible for a significant amount of economic loss to the sheep industry in Wales. Indeed nearly 30 per cent of the incidents recorded between 1 January 1975 and 31 December 1988 were diagnosed at the Centres in Wales and some of those recorded by the Centres in England were in sheep which had originated from Wales. It is also believed that incidence of coenurosis in sheep and cattle could be significantly reduced if appropriate control measures were adopted by the farming community. Any control measures introduced for the control of coenurosis however, must be based on sound and practical scientific principles and this can only be achieved if the epizootiology of coenurosis is fully understood. Because of gaps in the knowledge of the epizootiology of the disease an investigative study was planned and executed with the following aims and objectives:-

- 1 Review the literature on coenurosis, particularly that on the epizootiology and pathogenesis

- 2 Assess the prevalence and importance of the disease in sheep flocks in Dyfed by a postal questionnaire survey
- 3 Determine the prevalence of subclinical coenurosis in fat lambs sent for slaughter
- 4 Determine the role of farm dogs, pet dogs, hounds and foxes as definitive hosts of Taenia multiceps and establish the relationship between cestode infection and food items in their diet
- 5 Study the shedding of proglottids in dogs experimentally infected with Taenia multiceps
- 6 Study the viability of oncospheres and coenuri of Taenia multiceps
- 7 Study the morphological features of Taenia multiceps and assess its validity as a species
- 8 Study the pathogenesis of the disease in experimentally infected lambs.

1.2 A review of the literature

Coenurosis is a disease mainly of sheep, goats and cattle which is characterised by locomotor disturbance. The causal agent is Coenurus cerebralis, the larval stage of the dog tapeworm Taenia multiceps, which develops in the brain or spinal cord and produces pathognomonic lesions.

A Historical review

There are few references in the literature before 1600 that can be construed as referring to coenurosis. There is no doubt however that the disease known to farmers and veterinarians as "gid" or "sturdy", and "bendro" in Wales, must have existed for thousands of years, even before primitive man passed from the hunting to the pastoral stage. When the ancestors of the present day dog pursued wild sheep and goats, the adaptation of a brain parasite that interfered with muscular activity and thus make escape difficult, furnished a life cycle that was well calculated to perpetuate the parasite. This arrangement was hardly more satisfactory than the new one evolved by man, when he domesticated the sheep and introduced the dog as an aid to shepherding, allowing it to roam freely over the pastures and eat discarded heads of healthy and diseased sheep.

The clinical signs of the disease are so characteristic that pastoral people like the Arabs, Greeks and Jews must have noted them and their literature in all probability must contain numerous references to the disease. One such reference occurs in Adam's translation of Kuhn's edition of Hippocrates who is believed to have lived from 400 to 375 BC (Hall 1910). This reference describes an excess of fluids on the brain in epilepsy:- "This you may ascertain in particular from beasts of the flock (ie sheep) which are seized with this disease and more especially goats, for they are most frequently attacked with it. If you will cut open the head you will find the brains humid, full of sweat and having a bad smell". Adams, who was a physician refers to the quotation as follows:- "It is well known that this is also the case with sheep and that they are subject to the disease called the sturdy (ie gid) which is indisputably a form of epilepsy".

The first authentic record of coenurosis appeared about 1600 (Hall 1910) and although there is a rather limited literature on helminthology between 1600 and 1800, the gid parasite figures increasingly. This increase was perhaps concomitant with an increase in the number and distribution of sheep and cases of gid or sturdy, as well as an increasing knowledge of the parasite and the disease.

Markham (1616) in his "Method or Epitome" refers to 12 diseases of cattle including gid, which could be cured by his medicine number 3, consisting of the "leaves of Aristoloch, lard, tallow and the ashes of an old shoe, turpentine tar and the root of a lily". He also adds that in gid the skull must be opened and the bladder removed, but does not specify how this delicate operation should be performed.

Scutletus (1672) recorded that he first saw a case of coenurosis on 24 December 1634 and described another encountered in 1645. In both instances he gives details of the position and size of the cysts and comments that the disease was common in Germany and was known colloqually as "wirbling".

John Crowshey (1636) published a book "The Countryman's Instructor" which was intended to be available for his friends in the counties of York and Lincoln, and in this volume there is an interesting account of "the turne, sturdy or giddy" in both cattle and sheep. He also described his surgical operation for the cure in some detail. Two incisions were made in the skin on either side of the soft area of the skull, which were connected by a horizontal incision below. The skin flap so formed was then raised and the bone over the cyst removed with a knife and hammer. A goose quill was then introduced into

the cranial cavity for the location and removal of the cyst, after which the skin was sutured.

It seems that coenurosis was not uncommon in Europe during the seventeenth century and Rolfnick (1656) referring to vertigo states that it is occasionally caused by "sacs of water on the brain of sheep". It was also regarded as a common disease of cattle in Switzerland by Wepfer (1658), who also describes the characteristic clinical signs, pathology and morphology of the water bladder. Furthermore he states that the disease is a frequent cause of death in cattle and gives an account of the surgical operation to remove the water bladder.

In the English literature of the time Michael Harward (1673) in his treatise "The Herdsman's Mate or Guide for Herdsmen", devotes a section to coenurosis. In this he describes the clinical signs and outlines an operation for the removal of the cyst, which he cautions should only be attempted when the forehead becomes soft and yielding. He warns however, that the cure is difficult when the "turn" (ie coenurus) is ripe, dangerous and desperate when not ripe, and too late when over ripe.

Towards the end of the seventeenth century and during the early part of the eighteenth, several European workers developed an interest in the disease. Brunner (1694)

quoted by Kuchenmeister (1880) dissected the head of a "giddy" calf and found in the cerebrum three cysts, the size of pigeons eggs, full of fluid. Kuchenmeister believed that these cysts were Coenurus cerebralis. In a report Wepfer (1724), put forward the hypothesis that cysts in the brains of cattle were the cause of vertigo. He had seen peasants perform an operation for their removal and had also seen them demonstrated after a post mortem examination. Gutebruk (1766) quoted by Kuchenmeister (1880) in a treatise on sheep diseases states that the "gid" attacks lambs and yearlings but not old sheep. He also states that some lambs are born with the disease and that a water bladder forms on the brain and may penetrate the skull. He recommends that if the disease has not gone too far, the flesh may be used and the head and feet should be thrown away, but if the disease has gone too far, the entire carcass should be discarded. His recommended treatment was venesection on the temple and nose.

In 1775 Stier published a review article on coenurosis, listing the alleged causes, but rejecting them all except for the water bladder in the head and he draws careful distinctions between coenurosis and simulated coenurosis due to the larvae of Oestrus ovis. Bloch (1780), Hastfer (1776) and Ranstler (1776) also published articles on coenurosis, but none of these attracted much attention.

It was not until 1780 that the cestode nature of the bladder in the brain of "giddy" sheep was established by Leske and later by Goeze (1782) who arrived at the same conclusion. Goeze was of the opinion that the heads on the coenurus wall were embryos of the bladderworms found in the omentum and liver of sheep and swine. Leske made a careful study of the morphology of the parasite and pathology of the disease and from that time strenuous efforts were made to determine the life history of the parasite and to evolve rational prophylactic measures.

James Hogg, the Ettrick Shepherd, published his Shepherd's Guide in 1807, devoting much attention to coenurosis, particularly the surgical treatment of the condition, an operation he had carried out in his youth in the following manner "... but as I was frequently knitting stockings, I fell upon the following plan: I caught every sturdied sheep that I could lay my hands upon and probed up the nostril to the brain with one of my wires. I beheld with no small degree of pleasure, that by the simple operation, I cured many sheep to different owners, but I kept all my projects to myself, for I had no authority to try my skill on any of them". In later years he modified his technique by just locating the cysts as a softening of the skull and introducing the wire in the direction of the cyst: "If the wire could be felt below the thumb then the operator may rest assured the bag is perforated and that if the

brain does not inflame the sheep will get better".

Mackenzie (1809) in a treatise on the diseases and management of sheep, deals with the operation for sturdy. He regards "the more delicate and nice operation of trepan and extraction of the cyst are fit to be in the hands of skilful surgeons. But with ordinary servants the bungling of either would be fatal, would occur so frequently that only the simple operation of wiring should be described". Then follows a detailed explanation of how the operation is carried out complete with diagrams, illustrating the anatomical features of the skull and brain. One refinement of Hogg's operation was the use of a trocar by Mackenzie. In 1837 Youatt published a book, "Sheep, Breeds, Management and Diseases", in which he reproduced Mackenzie's diagrams and surgical approach.

In 1844 von Siebold postulated that the bladderworms were cestode embryos which in attaining a new host, had gone astray, ending as encysted incompletely developed forms, a theory that was supported a year later by Dujardin (1845). The experimental work of von Siebold and Kuchenmeister published in 1853, clarified the situation and it was Kuchenmeister who succeeded in experimentally demonstrating for the first time, the entire life history of a cestode. He fed a Coenurus cerebralis cyst to a dog and produced a tapeworm which he called Taenia coenurus,

and when he fed the gravid proglottids of this tapeworm to a sheep and produced in it the early stages of the coenurus in the brain.

From this experimental work Kuchenmeister concluded that sheep are infected at pasture by dogs shedding proglottids. On the evidence from his work, Kuchenmeister formulated a set of rules for the control of coenurosis which were:-

- a) feed dry food all the year round,
- b) once or twice a year purge the sheep and dogs in an enclosed area to rid them of tapeworms and burn the faeces,
- c) do not throw the heads of giddy sheep to the dogs or throw the brain to the dogs before cooking the heads.

Kuchenmeister's work was confirmed at a number of European universities in the following year and much publicity was given to his work in Britain following the publication of an article in the Veterinarian (Anon 1853). As well as referring to Kuchenmeister's work this article also gives an account of the observations of Professor Zaugger of the Zurich Veterinary School on the gid parasite and on other cestodes of domestic animals. It is evident that a great deal of confusion over the identity of individual tapeworms existed at that time and it was assumed that one

parasite was capable of developing in different sites in different intermediate hosts.

The situation was not fully clarified until Hall (1910) published the result of his very careful and masterly observations on the gid parasite and allied species in the USA. He followed this up with another publication in 1920, in which he described the taenoid species of cats, dogs and related carnivores in North America. Hall's work firmly established that Taenia (Multiceps) multiceps was the cause of coenurosis in sheep and other herbivores.

B The taxonomy and morphology of Taenia multiceps
 (Leske 1780)

The taxonomy of the genera Multiceps and Taenia has been studied and discussed by a number of workers over a period of many years (Hall 1920, Cameron 1926, Southwell 1930, Sandground 1937, Clapham 1942, Wardle and McLeod 1952, Meyer 1955, Rausch and Williamson 1955, Yamaguti 1959). Despite the extensive studies and discussions, a great deal of controversy arose over the conclusions reached by some workers, because most of the characteristics used were not considered to be valid by others. Indeed Esch and Self (1965) were of the view that the comparative

sizes of the long and short rostellar hooks are the only reliable criteria for separating individual species, a view which attracted much support.

Leske (1780), because of the presence of many heads on the coenurus wall, considered that it should be called the many headed tapeworm or Taenia multiceps. Goeze (1782) divided what he considered to be the genus Taenia into two main classes: Taenia visceralis, the visceral tapeworms, and Taenia intestinalis, the intestinal tapeworms. In the former he lists among other species, Taenia vesicularis cerebrina from the brain of a "giddy" sheep and "because of the presence of many heads one may call the parasite "Multiceps". Stiles and Stevenson (1905) summed up the position at that time, by stating that most authors recognise that the genus Taenia "is to be subdivided into the subgenera Taenia, Multiceps and Echinococcus". Others however were inclined to regard the subgenera as of full generic rank.

Hall (1920), Wardle and McLeod (1952) and Yamaguti (1959) recognised the validity of the genera Taenia, Multiceps and Hydatigera (species with the larval stage as a strobilocercus), and defined the criteria for identifying Multiceps species, as the presence of sinuous handles on the large rostellar hooks, a reflexed loop in the vagina and the possession of a coenurus. Cameron (1926) and

Rausch and Williamson (1955) disagreed and argued that Multiceps should be suppressed as a synonym of Taenia. Esch and Self (1965) and Verster (1969) support this view, suggesting that the genus Taenia should include all relatively large cestodes, usually with a distinct rostellum, armed with a double row of alternating long and short hooks, a distinct neck, a straight or curved vagina in the vicinity of the genital atrium, with from ten to several hundred proglottids and a bladder type larva having a single or multiple scolices, but no daughter cysts.

Before 1942 the number of species in what was regarded as the subgenus Multiceps was thirteen. At this time Clapham (1942) drastically reduced the number of species to six, basing her identification on measurements of the large rostellar hooks and concluded that the species serialis, glomerulus, packi, spalacia, clarifer, polutuberculosis and ramosus were different strains of M. multiceps. She also suggested that because there were several strains or variants of Multiceps, this would explain the wide range observed in the species of intermediate hosts, and the differences in the sites of localisation of the larval stages in the intermediate hosts. Nagaty and Ezzat (1946), Soulsby (1968) and Verster (1969) do not agree with this view and consider that T. multiceps and T. serialis are different species on morphological grounds.

The morphology of T. multiceps has been well described by a number of workers since it was first described by Leske (1780). The most comprehensive description is that by Hall (1910, 1920) and the following description is based on his observations.

The mature worm is 46 - 100 cms in length and made up of 200 - 250 segments. The head is pyriform in lateral view and has a diameter of 800 μ . There is a double crown of 22 - 32 hooks on a weakly developed rostellum which is 300 μ in diameter. The large hooks are 150 - 170 μ long with a slightly curved blade, a straight handle which is notched along its distal end. The small hooks are 90 - 130 μ in length with a moderately curved blade, a long curved handle terminating in a narrow distal extremity and a guard which in profile is usually subcylindrical, but proximally conical at its distal end and slightly grooved. The four suckers have a diameter of 290 - 300 μ . There is a distinct neck 2 - 3 mm in length and the individual segments are thin and relatively translucent with a maximum width of 5 mm. The genital pores are distinct from about 4 - 7 cms from the head at approximately the eighteenth segment. The first mature segment can be identified 10 - 18 cms from the head and is usually about the 125th segment. Mature segments are oblong, longer than wide, but never wider than long, with slightly convex lateral margins. Usually there are between 12 and 23

gravid segments which are 6 - 11 mm in length and 3 - 5 mm in width. The calcareous corpuscles are very small, 15 - 16 μ in diameter. The longitudinal excretory canals are small, the ventral lying about 420 μ from the margin of the segment. Because of the translucency of the segments the genital papilla is quite distinct and rather flat.

There are approximately 200 testes in one horizontal plane, but they are confined to the lateral portions of the median field near the longitudinal canals. They do not however, extend close to the field of the vas deferens and vagina, and leave a fairly wide free field on each side of the longitudinal canals. The testes extend alongside the bialate ovary, but do not press close to it or to the vitellarium. The vas deferens originates close to the median stem of the uterus on the genital atrium side of the segment and extends in a series of loops to the cirrus pouch which is 315 - 350 μ long and 110 - 145 μ wide.

The ovary is rather elongated along the longitudinal axis of the proglottid and both lobes are more or less equal in size. The vitellarium is small triangular and well separated from the male and female gonads and the shell gland is very small. Initially the vagina follows the line of the vas deferens before curving around the nearest ovary to the receptaculum seminis in the outer ovarian

field. In gravid segments the median stem of the uterus has 9 - 26 lateral branches, which remain distinct and without tendency to fuse. The oncospheres are 29 - 37 μ in diameter and have an embryophore which is 4 μ thick.

Verster (1969) in a comprehensive study which led to the revision of the genus Taenia, listed the main morphological features of T. multiceps which, allowing for differences in the preparation of the specimens examined are broadly in agreement. These together with some of those listed by Hall (1910, 1920) are tabulated in Table 1. Verster (1969) draws attention to an important morphological feature which allows T. multiceps to be differentiated from Taenia serialis. In T. multiceps a muscular pad can be identified adjacent to the anterior wall of the vagina between the latter and the cirrus pouch, whereas T. serialis has a well developed sphincter.

Soulsby (1968) lists the definitive carnivorous hosts as the dog, fox, coyote and jackal but sheep, goats and cattle as the intermediate hosts. Hall (1920) extends the list of intermediate hosts to include horses, chamois, gazelle, antelope and man. Probably the first authentic record of Coenurus cerebralis in man was diagnosed in a Paris locksmith by Brumpt in 1913 (Grove 1990).

C Epizootiology

Although the life history of T. multiceps has been known for a hundred years or more, comparatively little work has been carried out on the epizootiology of coenurosis. Dogs, foxes, jackals and wolves are listed by authors as primary or definitive hosts of the tapeworm, but the importance of foxes, jackals and wolves as sources of infection for domestic livestock has not been fully assessed. Similarly a number of intermediate hosts have been listed, but the importance of these hosts, other than sheep, cattle and goats has not been fully evaluated. If the views of Clapham (1942) and Esch and Self (1965) on the existence of a multiplicity of strains of T. multiceps capable of infecting a range of intermediate hosts are valid, then it is possible that small rodents could act as intermediate hosts.

Erchov (1961) expressed a view that although carnivorous species other than the dog could act as definitive hosts of T. multiceps, the principal agent in the perpetuation of coenurosis in Russia is the dog, particularly sheepdogs in daily contact with sheep flocks. The same author states that infection is more common in dogs in sheep rearing areas, than in dogs in other areas. Ronzhina (1953) found that dogs under two years of age were commonly infected and that T. multiceps reaches sexual maturity in 41 - 47 days. The same author also

demonstrated that the adult worm lives in the dog's intestine for a period of 69 - 156 days and when the infestation is a heavy one, as many as 30 gravid segments are passed in the faeces each day.

In Great Britain Cook (1965) found that T. multiceps was fairly common in farm dogs in certain areas. In mid-Wales for instance 8 per cent of farm dogs were infected, but in contrast to Ronzhina (1953), he found infection in dogs of all ages. Cook found no dogs infected with T. multiceps in East Anglia, nor was he able to demonstrate T. multiceps infection in red and blue foxes examined from a number of counties in England and Wales. Edwards, Hackett and Herbert (1979a & b) examined farm and kennelled dogs, hounds and foxes in Snowdonia and although they found cestodes in dogs, hounds and foxes, concluded that the prevalence of cestodes including T. multiceps was relatively low compared with other areas of Wales.

The oncospheres of T. multiceps may remain viable on pasture for months, although the survival period is governed by environmental conditions. Abassov (1965) demonstrated that oncospheres were viable for a period of about two months on pasture when the air temperature fluctuated from -16°C to 10°C. Erchov (1961) found that oncospheres which had been completely and continuously covered by snow for 160 days were still viable.

There is little published information on the prevalence of coenurosis in farm animals. From the evidence available it seems that few sheep rearing countries are free from the disease. The successful hydatid eradication campaign in Iceland has also eradicated cestode infections in dogs, including T. multiceps infection, and the similar campaigns in New Zealand and Tasmania have reduced cestode infections in dogs to extremely low levels (B R Cook personal communication). Although coenurosis was recognised in the USA (Hall 1910, 1920) it no longer exists there (Becklund 1970), despite the fact that no special control programmes were launched in that country. In other countries the disease is well recognised by sheep farmers and veterinarians but there is no reliable information on incidence or indeed prevalence. Erchov (1961) reported a mortality of 1.98 per cent in one area of Russia, but this figure did not include those animals slaughtered during the early stage of the disease.

The viability of the coenurus in the tissues of the intermediate host after death is an important factor in the perpetuation of the disease. Abassov (1965) found that scolices harvested from a coenurus, kept at freezing temperatures (-3° to -19°C) for 1 - 3 days, 5 days and 10 days were non-viable. After burial in the soil for two days, scolices were still viable, but non viable after five days. Thus it seems that under field conditions, the

coenurus does not remain infective for a long period, which is in sharp contrast to oncospheres shed by the definitive host.

Whilst sheep, goats and cattle are recognised as the main intermediate hosts, a number of workers have attempted to demonstrate that rodents are capable of acting as intermediate hosts and sources of infection for dogs. Ronzhina (1953) in a series of experiments failed to infect rabbits and other small rodents with oncospheres of T. multiceps, so that on this evidence they are unlikely to be intermediate hosts under field conditions. There are certainly no reports of Coenurus cerebralis cysts in rabbits and rodents although Coenurus serialis cysts have been recognised in rabbits in Dyfed.

D Pathogenesis

Infection of the intermediate host by larval cestodes follows the ingestion of embryonated oncospheres, usually with herbage. Within a period of time the oncospheres hatch and the hexacanth embryo is released. Although differences exist in the chemical requirements for hatching of taeniid oncospheres, the sequence of events which occur for all species appear to be similar (Gemell and Macnamara 1972). These include a) dissolution of the outer embryophoral membrane, b) dissolution of the

outer embryophoral blocks and disintegration of the inner embryophoral membrane, c) chemical activation of the hexacanth embryo and d) mechanical and possibly chemical disruption of the oncospheral membrane with release of the embryo.

After liberation from the oncosphere, the hexacanth becomes very active and the hooks move in a violent manner, their movements being controlled by contractile fibres. The hexacanth then penetrates the intestinal mucosa by means of their hooks and a penetration gland situated at the anterior end. Although the time taken by the hexacanth of T. multiceps to penetrate the mucosa of the intestine of sheep and cattle is not known, it may be similar to that given for T. pisiformis in rodents by Silverman and Maneely (1965) which is 10 - 40 minutes. Heath (1971) demonstrated that the hexacanth embryos of E. granulosus, T. ovis and T. hydatigena penetrate through the top of the villi in the jejunal region of the sheep's small intestine and continue to migrate down the villi until a venule of sufficient size is reached, which they then penetrate. It is logical to assume therefore that the hexacanth embryos of T. multiceps behave in a similar manner.

The hexacanth after penetrating into the intestinal vascular system are transported first to the liver via the

portal vein and then disseminated throughout the body via the general circulation. However it is only those hexacanth s that reach the brain and spinal cord that usually develop. Although Baxter (1958) reported a coenurus resembling that of T. multiceps in the subcutaneous tissues of an ewe.

Hexacanth s reach the brain between the seventh and twelfth day after infection (Ronzhina and Borodulina 1956), and at first wander around the brain and spinal cord, producing migratory tracks, but later they come to rest and the development of the coenurus commences. In the vast majority of cases in sheep only one or two hexacanth s develop into a coenurus, the remainder presumably dying off. The rate of development of the coenurus depends to some extent on localisation. Between two and four weeks after arrival in the brain or spinal cord, the coenurus measures about 4 mm in diameter, and by about the eighth week it may be 2 cm in diameter, the scolices forming at this stage (Soulsby 1965). If the number of hexacanth s reaching the brain is high, the animal may succumb to an acute meningo-encephalitis within 12 - 21 days after infection.

A number of workers who have studied the development and longevity of larval cestodes in previously uninfected vertebrate hosts have emphasised that a) the number of

larvae which develop is not necessarily proportional to the number of embryonated oncospheres fed, b) larval deaths occur throughout the period of parasite development, c) inflammatory reactions are excessive in juxtaposition to dead or dying larvae and d) abnormal morphogenesis of the bladder wall and failure to form a scolex aulage are not uncommon (Gemell and Macnamara 1972). The pathogenicity of T. multiceps for sheep depends on a number of factors, but the two main groups of factors would appear to be host factors and parasite factors. Age, strain and sex of rodents have been incriminated as possible causes of variations in the establishment and subsequent development of T. taeniformis in rodents by Dow and Jarrett (1960), and Abassov (1965) considers that these factors are also important in the development of clinical coenurosis in sheep. It is generally recognised that young sheep and cattle are susceptible to coenurosis and only rarely are older animals affected.

Silverman (1954) demonstrated considerable variation in the behaviour of taeniid oncosphere batches, not only in oncospheres from different worms, but also in oncospheres from different segments of the same worm. Such variations were reflected in the ability of hexacanth to invade and develop in a given normal host. He also demonstrated a similar variation in oncospheres derived from different

strains of the same worm. Esch and Self (1965) and Esch (1967) suggested that strain differences may well account for variations in hosts and predilection sites successfully parasitised by T. multiceps (T. serialis), a view which had been put forward by Clapham in 1942. Abassov (1965) reported that the infectivity of hexacanth of T. multiceps was related to the environmental temperatures used for his experimental work. Bondareva, Boev and Sokolova (1960) carried out a series of experimental infections on 11 wild and domestic species of animals and found that apart from sheep, the animals most susceptible to cerebral localisation of the coenurus of T. multiceps were antelopes, gazelles, wild sheep, Siberian wild goats and domestic cattle in decreasing order of susceptibility. In these experiments, deer, donkeys and wild pigs were resistant to infection, although Soulsby (1965) states that horses are susceptible.

E Pathology

Despite the fact that coenurosis has a world wide distribution and has attracted a limited amount of research, there is a dearth of knowledge on the pathological aspects of the disease. Gallego (1930) and Singer (1931) described the lesions produced during the acute phase of the disease, but it was not until Fankhauser, Hinterman and Valette (1959), that a

comprehensive account of the pathological features of the acute, subacute and chronic forms of the disease were reported. The following account is based on these three publications.

The acute phase is characterised by a purulent meningo-encephalitis which is more marked on the dorsal surface of the cerebral hemispheres, around the optic chiasma and extending along the ventral aspect of the brain stem. The tracks produced as a result of the migration of the hexacanth, are readily seen by the naked eye, and coronal section of the cerebral hemispheres reveals further tracks extending from the sulci into the cerebral cortex. Although the cerebral hemispheres are mainly affected, tracks may also be seen in the cerebellum and in the region of the corpora quadrigemina. Histologically the brain tissue within the tracks is liquefied and the tracks are essentially a mass of oedematous tissue in which there are bands of degenerating leucocytes and fibrin. Surrounding the tracks there are bands of fibrin, erythrocytes and leucocytes, and more distantly bands of lymphocytes. The blood vessels surrounding the lesion are swollen and often show a proliferation of the adventitia. Within the tracks, degenerating hexacanth may be located but often the only recognisable remnants may be the hexacanth hooks. More remote from the lesion, blood vessels may show some

perivascular oedema and eosinophilic cuffing. Because of the massive disruption of the brain, tissue and damage to the vascular system of the brain areas remote from the tracks may show necrosis.

In the chronic phase, which is usually produced by the development of one hexacanth into a full blown Coenurus cerebralis, the tissue damage is essentially one of atrophy, produced by a gradually increasing space occupying lesion. The gross pathology of the chronic phase is easily recognised. In a significant proportion of cases the frontal bones of the skull are rarefied, not only over the coenurus, but often over both cerebral hemispheres. The coenurus may protrude through the cerebral cortex or may be covered by a thin layer of cerebral cortex in a distended hemisphere. When the coenurus occludes the cerebral aqueduct and thereby interferes with the flow of cerebro-spinal fluid, both hemispheres may be distended and atrophied. Histologically the lesions are unremarkable in that apart from atrophy and displacement of brain tissue the only other changes are the formation of a fibrous capsule infiltrated into eosinophils, lymphocytes and some giant cells around the coenurus.

F Imunology

The immunological aspects of coenurosis have received

comparatively little attention in comparison with the work carried out on the immunological aspects of hydatidosis and cysticercosis. Indeed most of the investigations have been directed towards the development of a diagnostic test for coenurosis.

It is well recognised that young cattle and sheep are more frequently affected than their adult counterparts. It has been assumed that this in part is due to an age immunity, but exposure to subclinical levels of T. multiceps may also be a factor. It has been demonstrated too, that there is a cross community between certain species of cestodes (Ismagilova 1958, Gemell 1966) and therefore immunity against T. multiceps infection in adult sheep and cattle could develop following exposure to other cestode species and/or the presence of larval forms of these species in their tissues.

Allergic skin tests with coenurus fluid as an allergen have been used as an aid to diagnosis for a number of years. The exact basis of the test had not been fully determined, but it would appear to be a Type 1, anaphylactic, reagin-dependent reaction (Coombs and Gell 1967, Roit 1971). Ismagilova (1958) produced purified antigens from both the coenurus fluid and protoscolices, and claimed that not only could he differentiate between coenurosis and cysticercosis, but also between "active"

and "passive" phases of coenurosis, when he used these antigens in skin tests. Soulsby (1965) however expressed the view that skin tests were of little value because of the cross reactions produced by other tissue cestodes. Attempts to use serological tests as an aid to the diagnosis of larval cestode infection in animals and man have been largely unsuccessful because of the cross reactions produced by different species (Gemell and Macnamara 1972). However in the literature prior to 1972, there was no specific reference to a serological test for coenurosis.

A brief review of the immunological aspects of T. multiceps infection would be incomplete if no reference is made to the possibility of immunising sheep, especially lambs against coenurosis. Pukhov, Zinichenko and Chernobaev (1953) produced a vaccine which was prepared from the coenurus wall and strobila of mature T. multiceps and when administered either orally or subcutaneously induced a high degree of immunity. The production and use of such a vaccine in Great Britain has been suggested by Edwards and Herbert (1982), but such a course of action raises a number of issues which will be considered in some detail in the general discussion.

G Control

Kuchenmeister (1853) formulated a set of rules for the control of coenurosis, which were basically sound at that time. He recommended that dogs should not be fed or gain access to uncooked brains or heads from affected sheep and that dogs and sheep should be purged once or twice a year. As a variety of purging agents were used at that time, some of which had little value as taenicides and the recommended frequency of purging would not maintain dogs cestode free, it is unlikely that this measure had any significant effect. Nevertheless preventing the definitive hosts from gaining access to heads and brains and regular treatment of farm dogs with efficient taenicides is the basis of control, although it is essential that as much epizootiological information as possible should be available so that a judgement can be made on the importance of other possible definitive hosts, eg hunting dogs, domestic pets and foxes. Such measures have been successful in the control of hydatid disease in man and animals in a number of countries in the world.

Since the early 1930's a series of campaigns have been launched in several regions of the USSR which have proved successful as reported by Erchov (1961). The control measures employed were those recommended by the Institute of Scientific Research of Kazakhstan and are:-

- a) Early isolation of diseased animals with improvement in their rearing diet, and surgical treatment or slaughter
- b) Improvement of the sanitary and veterinary services on the collective farms
- c) Measures to reduce the danger from dogs
 - 1. Repeated dehelminthisation up to 4 times a year, animals being tied up, put on a diet and all infected faeces destroyed. The drugs used were any of the following:- arecoline, camala, filisan extract of male fern or grains of gourd (pumpkin)
 - 2. Each dog to be licenced
 - 3. The number of dogs to be reduced to 2 or 3 per farm and stray dogs to be destroyed
- d) Education of farm workers.

Mortality from coenurosis was reduced from 1.98 per cent to 0.18 per cent in a period of two years following the introduction of these measures.

The use of vaccines in lambs could play a role in controlling the disease, but as indicated in the previous section certain factors must be considered before it could be introduced in this country.

H Public Health Aspects

A review of the literature would not be complete without reference to the public health implications of infection with T. multiceps. Templeton (1968) reported that 50 cases of human coenurosis infections were recorded in the literature and they had occurred in the Congo, England, France, Kenya, Ruanda, South Africa, Uganda and USA. Thirty two of these were in tropical Africa and in 30, infection manifested itself in a single nodule in either the subcutaneous tissues or conjunctival tissue, whilst in the other two a coenurus was present in the vitreous humour. In contrast to this 13 of the 18 cases reported from outside tropical Africa involved the central nervous system, two the vitreous humour and the remaining three were located in the subcutaneous tissues. Thus there is a very obvious difference in the pattern of localisation in the tissues of man in these geographical areas.

The apparent difference in tissue localisation is difficult to explain. One possible explanation is that different species of cestodes are involved but Proctor (1964) stresses the difficulty of positive identification of cerebral cysts because many are degenerate. Fain (1956), Venderick, Fain, Langi and van Halen (1964) classified their cases from Uganda and Ruanda as being due to T. brauni on epidemiological, morphological and clinical grounds. The cases reported by Raper and

Dockeray (1956) and Templeton (1968) were similar and were probably due to T. brauni.

The high incidence of nervous involvement in areas outside tropical Africa suggests that T. multiceps was the species responsible. There is no concrete evidence on this point, but if as Clapham (1942) and Esch and Self (1965) postulate that a number of strains of T. multiceps do exist within a wide range of intermediate hosts, then it is likely that coenurosis in the human cases were derived from T. multiceps.

More recently Dietz, Tome, Montanari, Benedetti and Scaglia (1983) reported a human case of cerebral coenurosis in Italy, which they considered to be due to T. multiceps.

An interesting feature of the human infections, is that most of those infected have been children and infection it is thought, followed fondling of the dogs, an observation which is well recognised in human hydatidosis.

| | Hall(1920) | Verster(1969) |
|----------------------------|------------|---------------|
| <hr/> | | |
| Diameter of scolex | 800 | 746 - 956 |
| Diameter of rostellum | 300 | 273 - 364 |
| Diameter of suckers | 290 - 300 | 200 - 273 |
| Number of hooks | 22 - 32 | 22 - 30 |
| Length of large hooks | 150 - 170 | 157 - 177 |
| Length of small hooks | 90 - 130 | 98 - 136 |
| Number of testes | 200 | 284 - 354 |
| Length of cirrus pouch | 315 - 350 | 226 - 306 |
| Width of cirrus pouch | 110 - 145 | 100 - 125 |
| Number of lateral branches | 9 - 26 | 12 - 15 |
| in uterus | | |

Table 1. The main morphological measurements (in microns) of Taenia multiceps as listed by Hall (1920) and Verster (1969)

2.1 Morphology of Taenia multiceps

Over a period of many years a great deal of controversy has existed, and indeed continues to exist, over the identification of many Taenia species, including T. multiceps. A brief description only of the main characteristic features are provided in standard works on veterinary parasitology, eg Soulsby (1965, 1968) but Hall (1920) gave a comprehensive and detailed description of T. multiceps and other Taenia species.

In view of the paucity of detailed morphological descriptions of T. multiceps recovered in Great Britain, it was decided that a number of specimens from the Dyfed area should be examined, their morphological features studied and compared with those described by other authors, and these would then form the basic criteria for confirmation of the identity of suspected T. multiceps specimens recovered from dogs and hounds.

MATERIALS AND METHODS

A total of 12 adult specimens of T. multiceps and protoscolices from three coenuri (Coenurus cerebralis) were examined in detail.

The adult specimens were obtained from a five month old Border Collie cross puppy, which had been administered 20 protoscolices from a fresh Coenurus cerebralis removed from the brain of yearling ewe. Cestarsol at the recommended dose rate was administered 46 days after experimental infection and the 12 specimens were recovered from the purged faeces. Each cestode was carefully and gently washed in warm water and then warm saline to remove all traces of faecal material, its total length measured and some of the gravid segments removed for harvesting ova, destined for the experimental infection of lambs, rabbits and mice.

Individual worms were laid flat between two sheets of plate glass and then immersed in a bath containing 10 per cent formol saline for a period of 10 days. After fixation the scolices were then removed from each of the fixed cestodes, mounted in 90 per cent phenol and examined microscopically. The general features of the scolex were noted and the diameter of scolex, rostellum and suckers obtained by means of a calibrated ocular micrometer. The number and arrangement of the rostellar hooks were also noted. Five large and five small hooks were then removed from the scolices with a fine scalpel and forceps and their length and shape recorded. Mature and gravid

segments from each specimen were embedded in paraffin wax, sections cut at 5, 10 and 15 μ , stained with haematoxylin and eosin and examined microscopically.

The volume of the three coenuri removed from the brain of the infected sheep were estimated by puncturing the cysts and collecting the cyst fluid into an appropriately sized measuring cylinder. The number of scolices were counted with the aid of a hand held magnifying glass and the arrangement of the scolices on the coenurus wall noted. Five protoscolices were removed from each coenurus and examined in a similar manner to the scolices removed from the adult cestodes.

RESULTS

Some of the main morphological features are illustrated in Figs 1 and 2.

The lengths of the 12 adult cestodes are set out in Table 1. All specimens were gravid and their lengths varied from 42 to 61 cms. However the length of individual specimens was not dependent upon the number of gravid segments, which varied from five to twelve.

The scolex of the adult parasites was pyriform in shape and ranged from 750 - 900 μ in diameter (Table 3). The rostellum was not very prominent, 275 - 340 μ in diameter with 24 - 30 hooks arranged in two crowns and the suckers were 250 - 280 μ in diameter. There were 12 - 16 large hooks and the number of small hooks in the range 10 - 14. Large hooks were 154 - 177 μ in length and the small hooks 98 - 134 μ . The mean length and standard deviation of the large and small hooks are set out in Table 2. In addition to variations in the length of both large and small hooks there were also variations in their shape. The diameter of the suckers ranged from 250 - 280 μ .

The adult worms had a distinct neck of 1 - 3 mm. In both fresh and fixed specimens, the individual segments had a characteristic shape and appearance. The anterior segments were barrel shaped, wider than long with a slightly translucent border. Mature segments had a more pronounced barrel shape, longer than wide. Fresh mature segments were 4.5 - 6.0 mm long by about 3 mm wide with obvious translucent borders. Gravid segments were of a similar shape and size, but with a chalky white appearance. The genital pore was prominent in both mature and gravid segments located either midway along the border or slightly posterior but never in the posterior third, and its location varied irregularly from side to side in individual segments.

Within the proglottid the number of testes varied from 250 - 400, arranged in two lateral folds. The vas deferens was coiled throughout its entire length and the cirrus sac extended as far as the longitudinal canals, but not beyond, was 300 - 350 by 100 - 150 μ in the mature and gravid segments. A number of hair like structures or bristles covered the cirrus.

The ovary consisted of two lobes of similar size and the vitellarium was triangular in shape, located in a central position, posterior to the two ovarian lobes. There were 10 - 20 lateral branches of the uterus, which were further subdivided. The oncospheres were oval 26 - 27 μ by 23 - 33 μ with an embryophore 3.5 - 5.5 μ thick. In some of the sections examined a muscular collar or sphincter could be identified around the vagina, approximately 100 μ from the vaginal opening. The vagina tended to follow the line of the vas deferens initially, but then deviated towards the receptaculum seminis located between the two ovarian lobes.

The volume of the 3 coenuri was 35, 43 and 60 ml. Protoscolices were arranged in small clusters of 2 - 15, which were in turn parts of larger clusters (Fig 1h). The number of protoscolices counted on each coenurus was 311, 353 and 410.

The protoscolices closely resembled the scolices of the adult cestode and the diameters of the scolex, rostellum and suckers fell within the range of those found in the adults (Table 2). The arrangement, numbers of large and small hooks, their lengths and shape were similar to those for the adult specimens.

DISCUSSION

In her taxonomic revision of the genus Taenia, Verster (1969) asserts that the descriptions of many species are incomplete, thus leaving their status in doubt. She also claims that other species have been differentiated from existing ones on the basis of characters which are invalid. For instance descriptions are based on the assumption that cestode fragments recovered from the same host represent a single species, whereas subsequent work has shown them to be fragments of two or more species parasitising the host simultaneously. She found that in her work on the taxonomic revision of the genus, most of the characters used for specific identification are subject to some variation and that it is rarely possible to use a single character as the only criterion for specific diagnosis. The size and shape of the strobila, scolex, rostellum and suckers as well as the presence or absence of a neck for instance are dependent on the method of fixation and are thus invalid criteria. However she

agrees that the number and size of the rostellar hooks are reliable criteria, but in the case of small differences should be used in conjunction with other characters. Furthermore she states that the size of these structures should be determined on rostellum which are mounted "en face" and only those which are in profile measured.

In the present study every effort was made to avoid the criticisms and pitfalls highlighted by Verster (1969). The adult specimens were derived from dogs infected with one species only and were prepared and examined in exactly the same manner.

Taenia multiceps was first recognised and described by Leske in 1780 and subsequently considerable discussion and argument has ensued over the identity of the adult cestode. A number of authors (Clapham 1942, Dolfus 1959, Esch and Self 1965) have expressed the view that T. multiceps could not on morphological grounds be differentiated from T. serialis. Others including Ezzat (1946), Cook (1965) and Verster (1969) consider that on morphological grounds the two species are distinct.

The present morphological study although limited in extent confirmed some of the observations made by other workers. The overall lengths of the adult specimens fell within the range given by other authors (Hall 1920, Soulsby 1965,

1982). It must be emphasised however that the lengths of individual worms depends to a large extent on the weight of infection, the heavier the infection the greater the variation in length due to the crowding phenomenon (Bailey 1972).

Hall (1920) and Cook (1965) stated that the mature and gravid segments of T. multiceps are barrel shaped and longer than wide. Whilst this is evident in freshly voided specimens it is less so in preserved specimens. Hall (1920) claims that the proglottids of T. serialis are also barrel shaped but they are broader than long. Comparison of the proglottids of T. multiceps with those of T. serialis in the present study did not confirm the observations of Hall (1920). In fact the two species could not be distinguished on the shape of the proglottids alone in fresh specimens, a view which Cook (1965) also shares. The mature segments of T. multiceps have a markedly translucent border, a characteristic which T. serialis also shares according to Cook (1965). In the present study, translucence of the proglottid border was evident in both T. multiceps and T. serialis. The genital pore was easily recognised especially in the mature proglottids and was at or near the middle of the proglottid compared with T. serialis, in which it was usually situated in the posterior third of the proglottid.

The genitalia in T. multiceps were as broadly described by Hall (1920) and Verster (1969). However, Verster considers that an important diagnostic feature is the presence of a muscular pad on the anterior wall of the vagina in T. multiceps, which distinguishes it from T. serialis where the pad is replaced by a sphincter. In contrast none of the 12 adult specimens of T. multiceps examined in the present study had a pad, but in about half of the sections, a muscular band or sphincter could be identified surrounding the vagina.

In view of this evidence the assistance of Mr S Prudhoe of the British Museum (Natural History) was sought. Specimens of adult T. multiceps and T. serialis recovered from experimentally infected dogs, were submitted to him. He confirmed the identity of the two species submitted, but found a sphincter around the vagina in T. multiceps but a muscular pad attached to the anterior margin of the vagina in T. serialis. Correspondence between Mr Prudhoe and Dr Verster failed to resolve their differences in relation to the diagnostic features of the two species.

In 1982 further contact with the Parasitic Worm Section of the British Museum (Natural History), resulted in additional views being expressed on the presence of absence of a muscular pad/sphincter in T. multiceps. Dr R A Bray who had been involved in the earlier work on

identification, discussed this characteristic with Dr Arlene Jones of the Commonwealth Institute of Parasitology, who had carried out extensive observations on African taeniids. She had found the occurrence of this pad very inconsistent being present or absent in different segments of the same strobila. Dr Bray also indicated that Dr M Burt of the University of New Brunswick proposed initiating experimental work on the identity of T. multiceps and T. serialis but the results of this work has not yet been published.

The characteristics and dimensions of the scolex, rostellum, suckers and hooks of the adult specimens and 15 protoscolices examined were similar to those found by others notably Hall (1920), Dolfus (1959) and Verster (1969) and are listed in Table 3.

There were hundreds of protoscolices on the walls of the three coenuri. It has always been thought that the number of protoscolices increases with the age of the coenurus, that is the numbers increase when the coenurus increases in volume. One of the characteristics of Coenurus cerebralis is that the protoscolices are arranged in clusters and not in serial rows as in Coenurus serialis. Willis and Herbert (1987) describe a method for estimating the age of T. multiceps coenuri by counting the average number of scolices in each cluster and relating it to a

calculated regression line derived from an examination of coenuri of known age. These workers found that in experimental infections the number of protoscolices per cluster varied from 4.1 in a 2.1 ml cyst to 17.4 in a 60 ml cyst, whereas the total number of scolices were 37 and 419 respectively. Thus the total number of protoscolices in the three coenuri examined were similar to those recorded by Willis and Herbert (1987). However it should be emphasised that often it is difficult to firmly identify a cluster as some appear to be a part of a larger cluster. Therefore the total number of protoscolices may be a more accurate method of estimating the age of a coenurus, than the average number per cluster.

This study formed the basis upon which T. multiceps recovered from dogs, hounds and one fox were identified. Whereas a preliminary identification of T. multiceps and T. serialis could be made on the characteristics of the strobila, especially the translucent edges of the proglottids, final identification was based on the dimensions of scolex, rostellum, suckers, size and shape of the large and small hooks.

| Specimen number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Average length |
|-----------------|----|------|----|----|----|------|----|----|----|------|----|----|----------------|
| Length of worms | 53 | 47.5 | 42 | 45 | 49 | 51.5 | 60 | 61 | 54 | 51.5 | 46 | 49 | 50.79 |

Table 1 The length in centimetres of 12 adult specimens of T. multiceps recovered from an experimentally infective dog

| Source | Large hook | | | Small hook | | |
|------------|------------|--------------|-----------------|------------|--------------|-----------------|
| | No exam | Length range | Mean \pm SD | No exam | Length range | Mean \pm SD |
| Coenurus | 75 | 152-175 | 165.2 \pm 4.9 | 75 | 95-132 | 123.6 \pm 5.0 |
| Adult worm | 60 | 154-177 | 167.4 \pm 5.1 | 60 | 98-134 | 125.1 \pm 5.5 |
| Total | 135 | 152-177 | 166.3 \pm 6.0 | 135 | 95-134 | 134.5 \pm 5.3 |

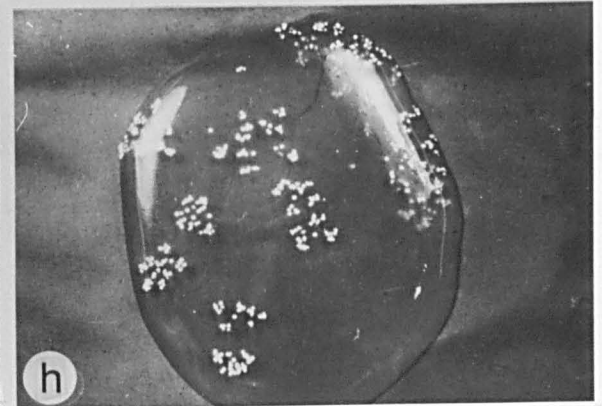
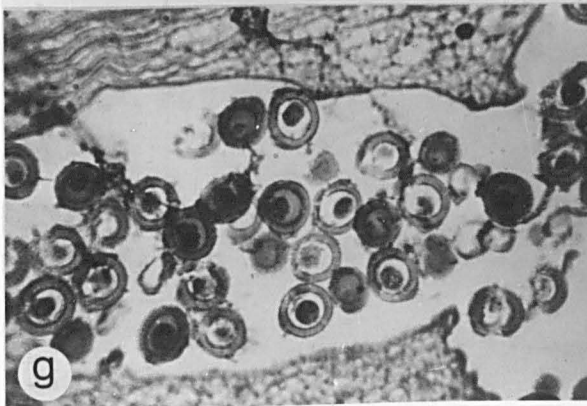
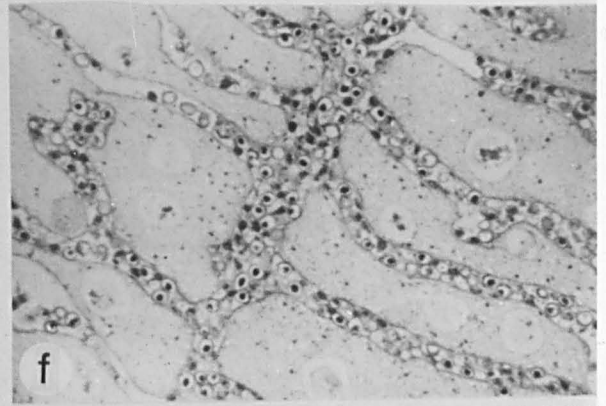
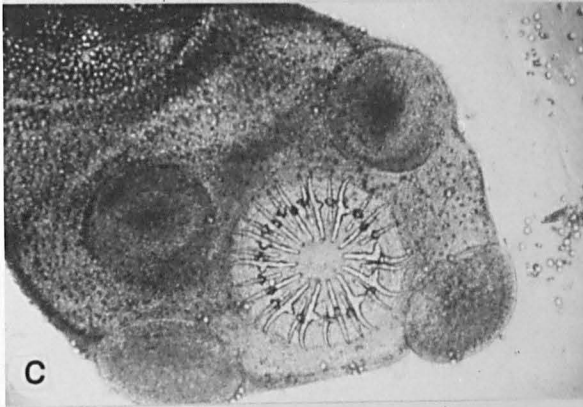
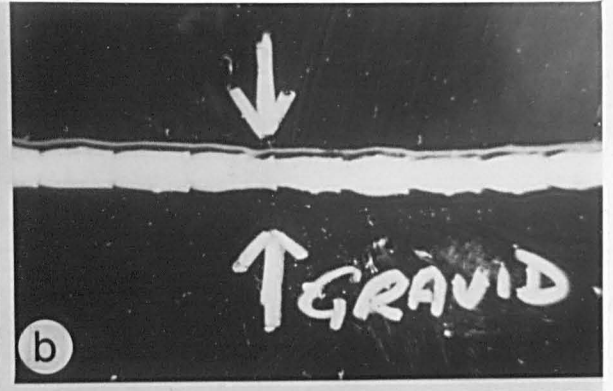
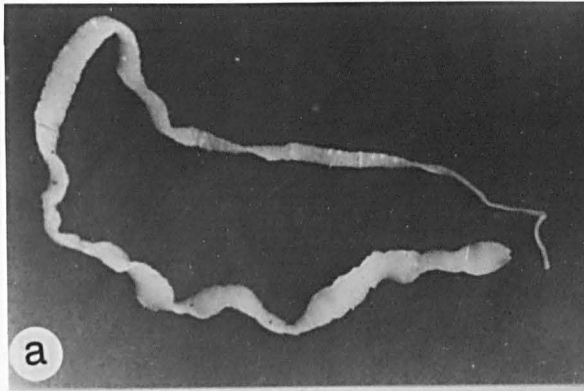
Table 2 Length in microns of large and small rostellar hooks from adult and larval specimens of T. multiceps

| | Hall 1920 | Dolfus 1959 | Verster 1969 | This paper |
|-----------------------|-----------|-------------|--------------|------------|
| Scolex | 300 | 750 | 746-956 | 750-900 |
| Rostellum | 300 | - | 273-364 | 275-340 |
| Suckers | 290-300 | 240-300 | 200-273 | 250-280 |
| No of hooks | 22-23 | 28 | 22-30 | 24-30 |
| Length of large hooks | 150-170 | 147-152.5 | 157-177 | 152-177 |
| Length of small hooks | 90-130 | 87.7-95 | 98-136 | 95-134 |

Table 3 Morphological measurements in microns of the scolex of T. multiceps according to various authors compared with those obtained in the present study

Fig 1 Morphological features of Taenia multiceps

- a) The mature cestode (x 0.2)
- b) A close up view of the strobila of the adult cestode, the seggments to the left of the arrow are mature and have a translucent border, those to the right are gravid and have a uniformly chalky white appearance (x 2)
- c) The scolex of the adult cestode, illustrating the double row of hooks on the rostellum and the four suckers (x 67)
- d) Typical large hooks (x 200)
- e) A typical small hook (x 400)
- f) The branched uterus in a longitudinal section of the adult cestode (H and E x 40)
- g) Transverse section of a gravid segment, illustrating the typical appearance of the oncosphere (H and E x 200)
- h) The intermediate stage; Coenurus cerebralis, the protoscolices are arranged in clusters on its wall (x 0.66)



3.1 Farm survey.

During evening meetings with groups of farmers, regular consultations with practising veterinary surgeons and periodic discussions with officials and representative of the Farmers Union of Wales and National Farmers Union, throughout 1970, 1971 and 1972, it became apparent that coenurosis was considered to be an increasing problem in sheep flocks. Requests for some form of action by the ministry (MAFF) to control or eradicate the disease was frequently voiced, particularly by flock masters from the Cilycwm and Rhandirmwyn area near Llandovery. It was claimed by farmers in these areas, that in their view coenurosis had become more prevalent since the construction of the Llyn Briane reservoir. Large numbers of visitors flocked to the area and a significant number brought their dogs which were then exercised over both common grazings and enclosed land on which sheep and cattle grazed. They also alleged that many dogs were either lost or deliberately abandoned during the summer months and consequently these dogs had to be either caught or shot to prevent sheep worrying.

Little information was available on the prevalence of coenurosis in sheep and cattle in the old county of Carmarthen. The Carmarthen Veterinary Investigation Centre had become operational on September 22 1969 and between that date and 31 December 1972 coenurosis had been confirmed by the laboratory in 51 sheep flocks and three cattle herds. Prior to September 1969, Cardiff Veterinary Investigation Centre was responsible for providing diagnostic services to the farming communities in the county, but because of the long distances which farmers had to transport live sheep and carcasses, few specimens had been submitted to the centre. Consequently apart from diagnoses based on clinical signs and the surgical removal of coenuri by practising veterinary surgeons and farmers there was no information available. Indeed because farmers frequently recognised the typical signs of chronic coenurosis, veterinary assistance was seldom sought, suspected cases were either operated upon by the farmer or were slaughtered and the carcasses either used for human consumption or fed to dogs.

Because of the dearth of reliable information available and the concern expressed by the farming community, it was decided to carry out a survey of sheep farms in Dyfed, in an attempt to ascertain the prevalence, and to collect information on the epizootiology of the disease. Because of the large number of sheep farms in the county it was

concluded that the most efficient and economical method of collecting this information was by a postal survey of farms in certain parishes.

Survey method

The divisional Executive Officer of MAFF, the person responsible for maintaining complete and up to date records of all livestock farms in the county, was asked to supply a list of sheep farms on a parish basis for the whole of the old county of Carmarthen. Those parishes with the highest sheep population were selected and a list compiled of the names and addresses of the sheep farmers in each parish. The lists were then submitted to veterinary surgeons practising in the area and they were asked to identify their clients and to give their permission for the clients to participate in the survey. In addition to the postal survey, farmers from other parishes who submitted live or dead sheep to the Carmarthen Veterinary Investigation Centre, were invited to complete the questionnaire.

Questionnaire

The questionnaire was drawn up as simply as possible to avoid any difficulties in its completion. Each farmer received a copy of the questionnaire together with a request that it be completed as fully as possible and returned within a period of six weeks in the prepaid

envelope provided. They were encouraged to discuss the questionnaire with their veterinary surgeons and if they wished to provide additional information which they considered relevant. Copies of the questionnaire and covering letter are at Appendix 10.1.

RESULTS

A total of 610 questionnaires were posted to farmers in January and February 1973 and 462 (75%) were returned by 30 April that year. A further 90 questionnaires were completed at the Centre between 5 January and 30 April 1973 by sheep farmers submitting specimens for laboratory examination. The participating farms are located in the parishes listed in Appendix 10.2.

1 Occurrence of Coenurosis

Coenurosis had been diagnosed on 375/552 (68%) of farms and on 331 (60%) it had been recorded for more than three years. On the remaining 44 (8%) the disease had been confirmed for the first time during the three years immediately preceding the survey ie, 1970, 1971 and 1972, although the flocks had been in existence for some years prior to that.

2 Losses due to Coenurosis

Annual losses recorded on affected farms varied from one or two a year up to a maximum of 40. Several noted that in some years losses were far higher than in others. One farmer reported that over a period of six years the annual losses were 3, 17, 32, 35, 40 and 6, but he could not account for this variation from year to year.

On a percentage basis the flock losses ranged from 0.5% to 29%, with an average of 2.5 - 3.0% per annum. Unfortunately because of the variable manner in which the information was recorded it is not possible to provide a more precise estimate.

3 Age of affected sheep

On the 375 farms where coenurosis was recorded, sheep under 12 months of age were affected on 293 (78%), over 12 months on 60 (16%) and in both age groups in the remaining 22 (6%).

4 Carcase disposal

Sheep carcasses were sent to knackeries by only 82/552 (14%) farmers and they were located within a distance of 3 - 5 miles of the knackeries. Even so, according to the farmers, carcasses were only collected when knackery vehicles passed the farm on their way to collect cattle or

horse carcasses from neighbouring farms. At other times sheep carcasses had to be transported to the knackeries by the farmers.

The majority of farmers, 301 (54%), sent carcasses to hunt kennels, the carcasses either being transported by the farmers in their own vehicles or collected by the kennel owners or employees. The remainder claimed that they either buried carcasses or occasionally burnt them. Several commented that during the summer months, when flocks were on more remote hill or upland grazings and shepherding was less frequent, carcasses by the time they were located had been attacked by predators and burial or other forms of disposal was not a necessity. Others drew attention to the fact that it was impossible to bury carcasses on some farms because of the shallow soil depth and if carcasses were buried they were subsequently exposed by dogs and foxes.

5 Number of dogs

There were 1,766 dogs kept on the 552 farms, an average of 3.2 dogs per farm, ranging in age from a few months to over 12 years. It later became apparent that in many instances pet dogs had been omitted from the return, so that the true total number of dogs was probably in excess of 2,000.

The normal age distribution given for the dogs on most farms was an old dog which had been retired or used for limited shepherding near the farmstead, a working dog between 3 and 8 years of age and a younger dog which was either being trained or worked with an older dog.

6 Feeding of uncooked meat and offal to dogs

Raw meat and offal were fed to dogs on 386/552 (68%) of farms. The replies indicated that this was deliberate policy and that often items from carcasses of dead sheep as well as those slaughtered were fed to dogs. Other farmers commented that it was impossible to prevent dogs from consuming uncooked meat and offal because although they did not feed such items to their dogs, they gained access to carcasses when they strayed from the farm or when they were used for shepherding on common grazings.

The relationship between the consumption of uncooked meat and offal by dogs and the recorded occurrence of gid in sheep is set out in Table 1.

Coenurosis occurred on 328/386 (85%) of farms on which dogs consumed raw meat and offal from sheep carcasses and slaughtered sheep. This was significantly higher than on 47/166 (28.3%) of farms on which dogs did not consume these items ($X^2 = 168$ $p < 0.001$).

7 Anthelmintic treatment of dogs

Dogs on 381/552 (69%) farms were never given treatment for tapeworms. On 92 farms (16%) dogs were treated at three monthly intervals or less. On the remaining 79 (14%) farms dogs were treated occasionally, usually at intervals of 6 - 12 months, but sometimes less frequently. No attempt was made to collect information on the type or brand of taeniocides used, because experience had shown over a period of 20 years or more that they obtained preparations from veterinary surgeons, pharmacists or travelling salesmen in small quantities, and were usually unaware of the identity of the preparations they obtained.

Information on the frequency of anthelmintic treatment of dogs was correlated with the occurrence of coenurosis in sheep in the same farms and this is set out in Table 2.

Of the 375 farms on which coenurosis occurred dogs were treated at 3 monthly intervals on 92 only and coenurosis was recorded on 23 (25%) only. Coenurosis was recorded on 32 (40.5%) of the 79 farms on which dogs were treated occasionally. In contrast of the 381 farms on which dogs were never treated, coenurosis was recorded on 320 (84%). Statistical analysis of this information indicated a significant reduction in the occurrence of coenurosis on farms where regular anthelmintic treatment of dogs was undertaken ($X^2 = 4.00$ $p < 0.05$) compared with that on

farms where dogs were only treated occasionally. The reduction in occurrence as compared to farms where dogs were not treated was much more significant ($X^2 = 168$ $p < 0.001$).

8 The relationship between anthelmintic treatment of dogs, the feeding of uncooked meat and offal to dogs and the occurrence of coenurosis in sheep

When the data on the anthelmintic treatment of dogs, the feeding of uncooked offal and meat to dogs, and the occurrence of coenurosis in sheep are considered together, the analysis produces a rather different picture as set out in Table 3. Although overall the results are significant ($X^2 = 128.23$ [df 5] $p < 0.001$) there are features which illustrate some of the complexities of the epizootiology of coenurosis. For example, the similar prevalence of coenurosis on farms where dogs were not treated irrespective of whether they were fed uncooked meat or offal or not (Table 3).

In the completed questionnaire a number of individual farmers made specific reference to factors outside their control, which were responsible for the occurrence of coenurosis on their farms. Such views had sometimes been expressed previously at meetings without any firm evidence being presented. Now 44 individuals from differing

locations offered firm evidence, that purchased ewe and ram lambs had either introduced coenurosis on to their farms or that the problem continued through such purchases. They further stated that coenurosis did not occur in their own home bred sheep. In addition four alleged that occasionally sheep returning from overwintering were affected, whereas those retained at home were not. Other theories, but no proof, for either the introduction or persistence, of infection included straying of dogs from other farms, abandoned pet dogs, passage of hounds over sheep pastures, exposure of sheep on common or open grazings and infected foxes.

When this evidence is considered, then on epizootiological grounds the data in Table 3 was amended, in Table 4. Thus of the 375 farms on which coenurosis was recorded in Tables 1, 2 and 3, the disease was endemic only on 331/552 (60%) which did not significantly alter the overall distribution of the farms in the various categories. However the proportion of farms on which endemic coenurosis was recorded, where no anthelmintic treatment of dogs was practised and no uncooked meat and offal fed to them was reduced from 99/118 (83.8%) (Table 3) to 62/118 (52.5%) (Table 4). Similarly the proportion of farms recording endemic coenurosis, where dogs were regularly treated and fed raw meat and offal was reduced from 19/70 (27.1%) (Table 3) to 15/70 (21.4%) (Table 4)

and from 9/26 (34.6%) (Table 3) to 6/26 (23.1%) (Table 4) on farms where dogs were occasionally treated and not fed uncooked meat and offal.

A more detailed analysis of the data in Table 4 revealed that when dogs were fed uncooked meat and offal, but treated regularly there was a significant reduction in the occurrence of coenurosis 15/70 (21.4%) in those farms as compared with 31/53 (58.5%) in those farms where only occasional treatment was administered ($X^2 = 17.705$ $p < 0.001$). There was a significant reduction also on those farms where occasional treatment was given to dogs, 31/53 (58.5%) as compared with those where dogs were never treated 213/263 (81%) ($X^2 = 11.614$ $p < 0.001$).

An analysis of the data on the 166 farms where no uncooked meat and offal was fed to dogs revealed no significant difference ($X^2 = 1.6325$ $p > 0.1$) between the proportion of farms with coenurosis where regular treatment 4/22 (18.2%) and occasional treatment 6/26 (23.1%) were practiced. However there was a significant difference ($X^2 = 7.4219$ $p < 0.01$) in the prevalence of coenurosis between farms where dogs were occasionally treated (6/26) and those where no treatment was given (62/118).

More detailed information on the food items consumed by dogs and the anthelmintic treatment of dogs on 100 sheep breeding farms included in the survey was collected during visits for this purpose and for treatment of dogs with Cestarsol to determine the prevalence of canine cestodes. This information is presented and related to the occurrence of coenurosis in Chapter 3.3.

Foxes

A total of 351 (63.5%) farmers stated that foxes were responsible for losses of lambs and also contributed to the spread of diseases including coenurosis. Indeed several asserted that between 1967 and 1972 there had been an increase in fox population in certain areas and this had coincided with an increase in the prevalence of coenurosis.

DISCUSSION

The proportion of completed questionnaires returned to the laboratory was surprisingly high and must have reflected to some extent the interest and concern of the farming community in coenurosis. It must be acknowledged that officials of the farming unions, especially one county secretary who was also a sheep farmer, and practising veterinary surgeons also played an important part in stimulating farmer interest.

Relatively few farmers keep detailed flock records, but they are required to maintain basic production records for completion of the Agricultural Census returns made in June and December each year. Therefore the replies were in the main based on estimates rather than precise records.

The survey confirmed the view that coenurosis in sheep was widely recognised in the area covered and the fact that it had been recognised in 60% of the flocks for more than three years indicated that in such flocks the disease was endemic. No specific reasons were given why coenurosis had recently appeared in some flocks which had been free of the disease for many years. However there was firm evidence given by 44 farmers that the purchase of ewe and ram lambs could well be an important factor in its introduction to certain flocks, but how common such an occurrence is responsible for its introduction nationally is a matter of conjecture. Two farmers independently identified farms from which they had purchased ewe lambs which developed coenurosis and subsequently the farms were identified as endemically infected. The movement of store lambs from Dyfed and Powys to Devon has been associated with outbreaks of cysticercosis, hydatidosis and coenurosis in sheep (I V Fincham personal communication) and more recently with outbreaks of coenurosis in cattle in that county. A small number of farmers also associated coenurosis with away wintering of ewe lambs, but it is

unlikely that this is a major factor, because older lambs and adult sheep are more resistant to infection than lambs under six months of age.

Whilst the survey disclosed that losses in some flocks from coenurosis were negligible in others they were not insignificant and could reach serious proportions. Most of the losses are attributable to the chronic form of the disease which is familiar to most farmers and they seldom seek veterinary advice. It is likely however that outbreaks of acute disease sometimes do occur and because of its nature, it may be mistaken for bacterial or viral meningo-encephalitis, or polioencephalomalacia and treated as such. Laboratory confirmation will only be sought when a significant number of lambs are affected. One such outbreak of acute disease was referred to the laboratory in 1972, in which 14 unweaned lambs developed nervous signs over a period of three weeks. All the affected lambs died and another six developed chronic coenurosis some months later. The one outbreak referred to earlier in this chapter, when 133 sheep were lost over a six year period represented a significant economic loss and caused disruption to the breeding policy on that farm. Outbreaks of a similar magnitude have been encountered in Powys and Dyfed by the author over a period of twenty years.

The survey confirmed that coenurosis affects sheep mainly in the first and second year of life. It has been generally accepted that infection is acquired during the first few months of life but in recent years evidence has emerged that older sheep can also become infected and develop clinical disease. Angelov and Belchov (1986) recorded two outbreaks of acute coenurosis affecting 50/180 adult sheep in one flock and 48/166 in another, two weeks after they were housed in buildings which previously housed dogs. Doherty, Bassett, Breathnach, Monaghan and McErlean (1989) also reported an acute outbreak of coenurosis in 150 ewes between 2 and 4 years of age in which 20 per cent developed clinical signs and 9 per cent died during the acute phase although it was not possible to determine the fate of ewes which recovered from the acute disease.

Carcase disposal on sheep farms, especially hill farms with grazing rights on common land presents considerable difficulties, which were highlighted in the survey. The reluctance of knackeries to collect sheep carcasses, because of poor economic return was understandable in view of the long distances between the knackeries and some of the farms. For instance collection of carcasses by the Cwm Ann knackery from farms in the Llandovery area would involve a round journey of more than 24 miles, whilst collection by the Abergwili knackery would involve a round

journey of 60 miles or more. Both of these knackeries would however collect sheep carcasses if cattle or horse carcasses were collected from the vicinity. In contrast knackeries in North Wales collected sheep carcasses within 24 hours at no cost (Stallbaumer 1985). In total only 14% of farmers sent sheep to a knackery and more often than not they transported the carcasses themselves.

Of the remaining 86% of farms, the majority relied on hunt kennels for carcase disposal. The survey area was served by ten kennels, four of which were run by Hunt Associations and the others by groups of farmers (Farmers' Packs). The kennels collected sheep and sometimes other animal carcasses, but because of their proximity many farmers were able to deliver carcasses to the kennels quickly.

Athough 149 farmers claimed that they disposed of carcasses through burial they stressed the difficulty of burial on certain farms where soil depth prevented satisfactory burial. In addition to this they emphasised that when sheep were on remote grazings and when shepherding was less frequent than on the home farm, sheep might have died 24 - 48 hours or even more prior to the discovery of a carcase, by which time there would be little left of the carcase because of scavenging by birds, foxes and dogs. Thus despite the provisions of the Dogs Act, which

requires farmers to dispose of carcasses promptly and safely it is not practical or possible to comply fully. A small number of farmers commented that when they had buried carcasses in presumably shallow pits, they had been quickly exhumed by dogs and foxes.

The most depressing feature of the survey, but not in the least surprising was the high proportion of dogs which consumed raw sheep meat and offal. Dogs on 68% of farms consumed such items which contrasts with 30% of farms in North Wales (Stallbaumer 1985). Stallbaumer also found a strong association between the feeding of uncooked meat and offal to dogs and coenurosis in sheep, which confirmed the association demonstrated in the present survey.

On only 32% (171/552) of farms were dogs treated for tapeworms, which contrasted with 72% of farms in North Wales reported by Stallbaumer (1985). Of the 171 farms regular treatment was practised on only 92 (17%) and occasional treatment on 79 (14%) farms. Stallbaumer (1985) recorded that 37% of farmers in North Wales treated their dogs at least once a year. The present survey demonstrated a clear relationship between treatment and the occurrence of coenurosis and this too was confirmed by Stallbaumer (1985).

Despite the claims by certain farmers that foxes had a role in the epizootiology of coenurosis, they provided no information to support such claims. There is no doubt that the fox population had increased to about one fox per hectare in certain parts of Dyfed (I B Jones personal communication) but in view of its behaviour and feeding habits it is unlikely to play a significant role in the epizootiology of the disease.

The survey clearly demonstrated that coenurosis was relatively common in Dyfed and despite the difficulties in making a precise estimate of its prevalence, losses were not insignificant. It also indicated that some aspects of the epizootiology required investigations in both field and laboratory, the results of which are reported in the following chapters.

| Diet of dogs | Number of farms | Number of farms on which coenurosis recorded | |
|--------------|-----------------|---|----|
| | | Yes | No |

| | | | |
|-------------------------|-----|-----------|----------|
| Dogs consuming raw meat | 386 | 328 (85%) | 58 (15%) |
|-------------------------|-----|-----------|----------|

| | | | |
|-----------------------------|-----|------------|-------------|
| Dogs not consuming raw meat | 166 | 47 (28.3%) | 119 (71.1%) |
|-----------------------------|-----|------------|-------------|

| | | | |
|-------|-----|-------------|--------------|
| Total | 552 | 375 (67.9%) | 177 (32.19%) |
|-------|-----|-------------|--------------|

Table 1 Association between the occurrence of coenurosis in sheep and the feeding of uncooked meat and offal to dogs on the survey farms ($X^2 = 168$ $p < 0.001$).

| Anthelmintic treatment of dogs | Number of farms | Number of farms on which coenurosis recorded | |
|---|-----------------|---|-------------|
| | | Yes | No |
| Dogs treated at 3 monthly intervals (a) | 92 | * 23 (25%) | 69 (75%) |
| Dogs treated ocassionally (b) | 79 | ** 32 (40.5%) | 47 (59.5%) |
| Dogs never treated (c) | 381 | 320 (84%) | 61 (16%) |
| Total | 552 | 375 (67.9%) | 177 (32.1%) |

Table 2 Association between anthelmintic treatment of dogs and occurrence of coenurosis on 552 sheep farms

* (a) v (b) $X^2 = 4.00$ $p < 0.5$

** (a) v (c) $X^2 = 66.5$ $p < 0.01$

| Diet and anthelmin- tic treatment of dogs | Number of farms on which coenu- rosis recorded (%) | Number of farms on which coenu- rosis recorded (%) | Total |
|---|---|---|-------|
| Regular treatment, raw meat and offal consumed | 19 (27.1) | 51 (72.9) | 70 |
| Regular treatment, no raw meat or offal consumed | 4 (18.2) | 18 (81.8) | 22 |
| Occasional treatment, raw meat and offal consumed | 31 (58.5) | 22 (41.5) | 53 |
| Occasional treatment, no raw meat of offal consumed | 9 (34.6) | 17 (65.4) | 26 |
| No treatment, raw meat and offal consumed | 213 (81) | 50 (19) | 263 |
| No treatment, no raw meat or offal consumed | 99 (83.5) | 19 (16.3) | 118 |
| Total | 375 (68) | 177 (32) | 552 |

Table 3 Association between anthelmintic treatment and feeding of dogs with the occurrence of coenurosis in sheep ($X^2 = 128.23$, $df\ 5$, $p < 0.001$)

| Diet and anthelmin- tic treatment of dogs | Number of farms on which coenu- rosis endemic (%) | Number of farms on which coenu- rosis not endem- ic (%) | Total |
|--|--|--|-------|
| Regular treatment, raw meat and offal consumed | 15 (21.4) | 55 (78.6) | 70 |
| Regular treatment, no raw meat and offal consumed | 4 (18.2) | 18 (18.8) | 22 |
| Occasional treatment, raw meat and offal consumed | 31 (58.5) | 22 (41.5) | 53 |
| Occasional treatment, no raw meat and offal consumed | 6 (23.1) | 20 (76.9) | 26 |
| No treatment, raw meat and offal consumed | 213 (81) | 50 (19) | 263 |
| No treatment, no raw meat and offal consumed | 62 (52.5) | 56 (47.5) | 118 |
| Total | 331 (60) | 221 (40) | 552 |

Table 4 Association between anthelmintic treatment and feeding of dogs with the occurrence of endemic coenurosis in sheep ($X^2 = 118.4368$, $df\ 5$, $p < 0.001$)

3.2 Coenurosis in lambs at slaughter

Coenurosis is essentially a disease of lambs with at least 80 per cent of recorded cases occurring in animals during their first year of life (B M Williams unpublished data). It is also known that the acute form of the disease is seldom seen or recognised as such and the chronic form develops slowly over a period of months. It seemed likely therefore that some of the healthy fat lambs sent for slaughter would show evidence of infection by the larval stage of Taenia multiceps (Coenurus cerebralis) and to this end a survey of lambs from Llandovery and the surrounding area was arranged.

MATERIALS AND METHODS

A proportion of the lambs from Llandovery and the surrounding area were slaughtered at the Builth Wells abattoir and with the full co-operation of the management and meat inspectors a survey was planned and carried out. Five hundred heads were selected by the meat inspectors from lambs known to have originated from flocks in the Cilgwyn and Llanfair-ar-y bryn parishes during September, October and November 1974 and another 500 were selected the following year during the same period. The heads were not identified with any individual farm.

All the lambs were electrically stunned prior to bleeding and consequently the brains were not damaged during the slaughter process. The heads were removed at the occipito atlantal joint, placed in a plastic bag and held at 4°C in a cold store until collected for transmission to the laboratory. Heads from lambs slaughtered on Monday, Tuesday and Wednesday were collected late on Wednesday. On receipt the heads were skinned and the brains exposed by making two cuts with a bone saw from a point immediately dorsal to the occipital condyles to the midline just below the level of the eyes, inserting a bone wedge into the saw cuts and prising the roof of the cranium upwards. The dura mater and arachnoid were then removed with a scalpel and forceps, and the head with the exposed brain in situ immersed in 10 per cent formol saline for a period of three to five days until the brain hardened to facilitate further examination.

Following immersion in formol saline the brain was removed from the skull and its surface examined visually and by palpation for the presence of coenurus cysts. The cerebral hemispheres and cerebellum were then sliced transversely with a sharp ham knife at intervals of approximately 2 mm and the cut surfaces examined for evidence of infection. The mid brain and medulla oblongata were not sectioned as the presence of any lesion in these areas would have produced clinical signs.

If recognisable cysts were located no further examinations were undertaken. Any suspicious or unidentifiable lesions were however subjected to a histological examination. The tissue was returned into formol saline to ensure complete fixation, then dehydrated embedded in paraffin wax and sections 10 - 15 μ cut and stained with haematoxylin and eosin. The stained sections were then examined microscopically and any necrotic lesion which was either infiltrated by or surrounded by eosinophils, with or without a degenerating parasite was classified as Coenurosis.

RESULTS

Of the 1000 heads collected and examined as described, 58 (5.8%) showed evidence of infection. Twenty six infected heads were found in 1974 and 32 in the following year. Recognisable cysts were found in 42 brains, either superficially on the cerebral surface or deeper in the cerebral cortex. They varied in size from 2 - 4 mm in diameter, although one cyst in the olfactory lobe of the cerebrum of one brain measured 7 mm in diameter. One cyst of 2 mm diameter floated in the lateral ventricle of another brain.

In a further 14 brains evidence of tracking, usually one or two yellowish purulent tracks on cerebral sulci or a diffuse but mild meningitis were found (Fig 1). Single necrotic lesions characteristic of parasitic infection were demonstrated in a further two brains.

DISCUSSION

The high rate of sub-clinical infection found in this survey was unexpected, particularly in view of the relatively low level (1.8 per cent) found by Cook (1965) at the same abattoir. It was not clear whether there had been a true increase in the prevalence of coenurosis during the intervening years as claimed by some veterinarians and farmers. It is difficult to compare Cook's survey with the present one. Cook only examined 126 heads and does not provide any information on the origin or ages of the sheep he examined. Furthermore the method he used for exposing the brain - "a series of blows with a common wood hatchet", together with the fact that the brains were not fixed in formol saline, is likely to have led to an underestimate of the prevalence of coenurosis at that time. In the present survey, 16 (27 per cent) of the confirmed lesions of coenurosis would have been missed if the brains had not been subjected to a histological examination.

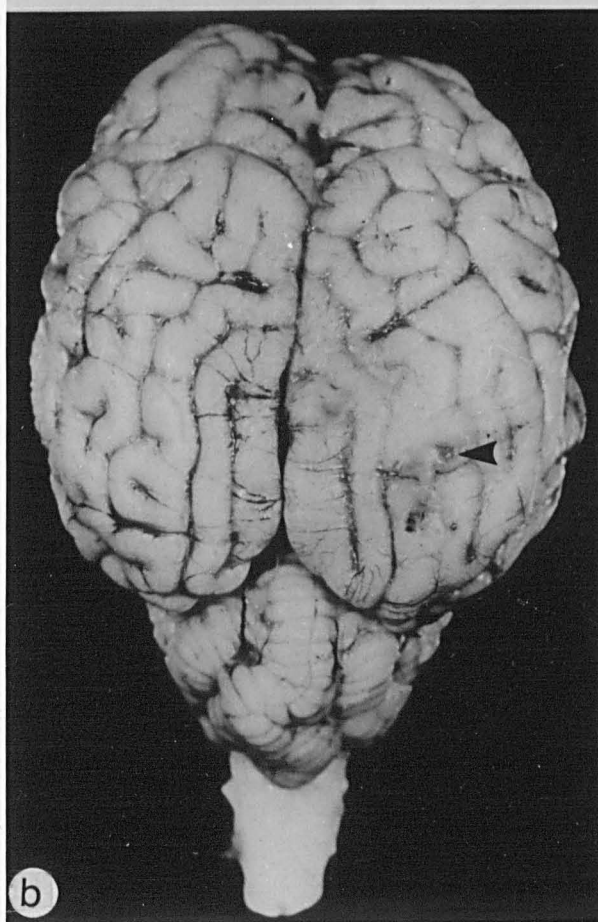
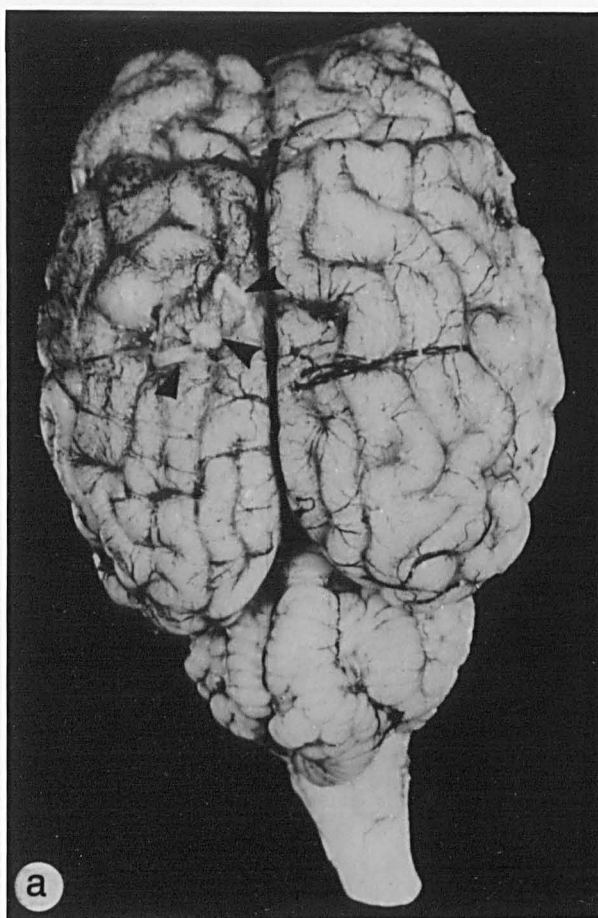
The significance of the prevalence rate in this survey is important, because undoubtedly a proportion if not all of the 42 lambs with identifiable cysts would have developed into clinical cases had they been retained in their flocks of origin. The meat inspectors at the abattoir reported that in the two three-month periods 110 sheep had been submitted for "casualty" slaughter and that a high proportion of these were clinically affected with coenurosis.

A number of farmers and wholesale butchers collected heads from the abattoir which were specifically intended for feeding to dogs. The majority of heads however were destined for meat processors in the West Midlands, but it was not known whether some were subsequently sold for feeding to dogs. It was likely therefore that some heads containing Coenurus cerebralis cyst from healthy slaughtered sheep and from "casualties" would be fed to farm dogs. Stallbaumer (1985) in a study of the epidemiology and prevalence of cestodes in farm dogs in Clwyd concluded that the feeding of sheep heads to farm dogs was a fundamental link in the life cycle of T. multiceps in that area. She also stated that the sale of heads was not economically important to sheep owners and a ban on the sale of heads would not be unpopular. It should be pointed out however that sheep sent for slaughter are either graded on the hoof and/or sold by

dead weight and the farmer selling the sheep is not penalised for any condemnations or rejection of viscera or other organs.

The survey therefore confirmed the information obtained from the questionnaire survey that coenurosis was relatively common in the two parishes. It also illustrated the need for and the value of such a survey in a study of the epizootiology of coenurosis.

- Fig 1 Evidence of infection by the larval stages of Taenia multiceps in the brains of lambs sent for slaughter
- a) Three tracks (arrowhead) on the left cerebral hemispheres (x 1)
 - b) A small nodule (arrowhead) and a slight, but diffuse meningitis on the surface of the right cerebral hemisphere (x 1)



3.3 The feeding and management of farm dogs

Larval or tissue cestodes of farm livestock, and indeed man are derived from cestode infected dogs and other carnivorous species. The information obtained from flock owners and shepherds during the farm survey (Chapter 3.1) revealed that a significant proportion of dogs consumed raw sheep meat and offal. Cook (1965) clearly demonstrated a relationship between the prevalence of hydatidosis in sheep and the consumption of raw sheep meat and offal by farm dogs in several areas of Great Britain, including mid and South Wales, but no such relationship had been established for ovine coenurosis. Thus it was decided to investigate this possible relationship by collecting information on the feeding and management of dogs on selected farms and to examine them for evidence of parasitism by cestodes. This chapter is devoted to feeding and management only.

METHOD

After consultations with practising veterinary surgeons and officials of the Farmers Union of Wales and the National Farmers Union it was decided to invite owners of sheep breeding flocks in the upper Cothi and Tywi valleys who had returned to the laboratory completed questionnaires during the farm survey, to participate in the investigation. Of the 111 owners invited to

participate 100 agreed to do so. In addition dairy farmers in the St Clears and Whitland areas, who provided grazing for sheep hill flocks during the winter months were also invited to participate. Eighteen agreed, all of whom confirmed that coenurosis had been diagnosed in overwintered sheep whilst on their premises. The majority of breeding farms were located in the Cilycwm and Rhandirmwyn area (Fig 1).

Information on feeding and management of dogs was collected during visits made to treat dogs with the taenicide Cestarsol and the collection of purged faeces samples. The details requested from flock owners covered the frequency of feeding, dietary items, whether dogs were tethered, confined or allowed to roam freely and the type and frequency of anthelmintic treatment. At the same time an opportunity was taken to clarify or amend statements which had been made in the completed questionnaire.

RESULTS

Frequency of feeding Just over 90% of adult dogs were fed once daily, usually late evening between 20.00 and 21.00 hrs. The remainder received a small feed of household scraps either early in the morning or at midday, as well as a main feed in the evening.

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The small number of farmers who bred from sheep dogs reported that weaned puppies up to the age of six months received three feeds a day, but from that age until they reached nine months, they were fed twice daily. Dogs over nine months of age were treated as adults.

Food items The main categories of food items on both the sheep breeding and wintering farms are summarised in Table 1.

The main constituent in the diet of over 80 per cent of the dogs was either flaked maize or maize meal, often moistened with either water or milk, or oatmeal porridge. The latter was usually prepared once or twice a week and stored in a large cast iron pot or an aluminium or cast iron saucepan and ladled out as required. During the winter months, the porridge would be reheated before feeding, especially when weather conditions were severe. On 30/118 farms this was the only food provided although occasionally it was supplemented by household scraps, which included cooked or uncooked bacon fat and rind,

trimmings from cooked and uncooked meats bought for human consumption, stale and toasted bread, cooked potatoes and other vegetables.

Rather surprisingly 55 per cent of dogs received milk; most of it fed deliberately although some owners reported that dogs managed to consume milk from calf buckets or from spillage in the dairy or milking parlour.

Only 15 per cent of dogs consumed dog biscuits or meal. Most farmers considered that the cost of these products was prohibitive and they were of the view that flaked maize, maize meal and porridge were more than adequate substitutes.

Canned meat was seldom purchased and only 7 per cent of dogs received any. Cost was the main reason for not feeding it, even when bought in bulk, and farmers argued that this outweighed convenience and ease of storage.

Collection of information on the feeding of uncooked meat and offal was frequently difficult, because it was related to whether dogs were confined or not during the day and night. Information on the purchase of meat, offal and bones from butchers specifically for feeding to dogs was more readily obtained and only 6 per cent of dogs were fed such items.

Thirty seven per cent of dogs consumed either occasionally or regularly uncooked meat and offal from sheep, usually lambs, sometimes wethers, slaughtered for human consumption either on the farm or at a local abattoir. The meat consisted of neck muscle trimmings and skirt (diaphragm). Heads were regularly returned with the carcass from the abattoir and thrown to the dogs. Dogs also received the lungs, melts (omentum), trimmings from the liver and occasionally paunches (the stomachs). Such practices were followed on 47 (40%) of the farms and in 12 of these, it was admitted that a sheep showing early signs of chronic coenurosis, would if in a suitable condition be slaughtered for human consumption and the offal and head fed to dogs on the farm.

It was standard practice on 41 (35%) of farms for dogs either to consume meat and offal obtained from the carcasses of dead sheep or they were allowed to scavenge on carcasses that had been skinned. However farmers insisted that only carcasses of recently dead sheep were treated in this manner and only when carcasses could not be collected or transported to knackeries or kennels, because of a glut of carcasses at the knackeries and kennels, or because of pressure of work on the farm. Although it was admitted by some owners and shepherds that dogs strayed from farms, they were confident that none of their own scavenged, a confidence that appeared to be misplaced in view of the

observations made by officials of the farming unions, several farmers and members of the public and the author's own experience. A total of 35% of dogs consumed uncooked material, either by scavenging or through being deliberately fed such items.

Thus 72% of dogs consumed raw meat, offal and heads from sheep slaughtered for human consumption or from carcasses of dead sheep. On a farm basis dogs on 76/100 breeding farms and 16/18 wintering farms consumed these items either regularly or occasionally. Table 2 sets out the relationship between the occurrence of endemic coenurosis on 100 sheep farms where dogs consumed or did not consume raw meat, offal and heads. There was a significant ($X^2 = 6.0893$ $p < 0.02$) relationship between the feeding of raw meat, offal and heads with the occurrence of coenurosis.

As far as the wintering farms were concerned, no statistical analysis was carried out because in the vast majority of instances, infection in overwintered sheep is acquired on the home farms.

Confinement of dogs Few farms provided purpose built accommodation for dogs and most were housed in barns, disused cowsheds and stables, implement sheds, dilapidated unused pig sties and chicken houses, and wooden barrels or oil drums laid on their sides. In some instances dogs

either by tethering or within buildings when not required for herding sheep or cattle, on only 15 farms, but 93 farms claimed that the dogs were either confined to building or tethered during the night.

Most owners and shepherds admitted that dogs tended to stray unless they were restrained in some way, especially during the summer and autumn when shepherding was less intense. Male dogs strayed oftener and over longer distances than bitches, but according to the owners and shepherds, dogs were seldom away for more than four to six hours unless a bitch on a neighbouring farm or house was in season. One owner claimed that one of his dogs was so intelligent that he had outwitted everyone on the farm. Although it was tethered during the night, it often slipped its collar, and would be seen some distance away by neighbours, yet the following morning it would be tethered to an oil drum as usual! To avoid any accusations of sheep worrying the dog was now confined to a barn at night.

The standard of accommodation was generally abysmal and provided little comfort or cleanliness for the dogs. Indeed this may have been a factor in the straying of some dogs. Only about a third were provided regularly with a clean straw bed or similar bedding. Some slept on earth

floors and some buildings particularly the dilapidated pig sties and chicken houses had leaking roofs which rendered them totally unsuitable. In others there was a build up of dirt and ammonia and if dogs were confined for long periods a build up of faecal material.

Anthelmintic treatment On only 17/118 (14%) of farms were dogs dosed at regular intervals of three to four months. Dogs on another 32 (27%) farms were treated occasionally, the majority about once a year and others when tapeworm segments were seen in the faeces.

A variety of anthelmintics were used for treatment and included bunamidine hydrochloride, dichlorophen and arecoline/acetarsol. The anthelmintics were obtained as required from pharmacists, agricultural merchants and less frequently veterinary surgeons. It is unfortunate that contact between sheep farmers and their veterinary surgeons is limited and in most cases is restricted to a few weeks around lambing time. Thus anthelmintics for dogs were in the main obtained from the other sources, so that limited advice on choice and use of anthelmintics were provided. A few tablets or capsules were purchased and these were dispensed in envelopes or bottles labelled "For animal treatment only. Worming tablets" with instructions for administration. When questioned only seven farmers knew the identity of the anthelmintics they

seven farmers knew the identity of the anthelmintics they administered. Identification of the range of anthelmintics used was confirmed by markings or inscriptions on the tablets when these were available for identification.

It transpired during discussion that many farmers were unaware that only certain anthelmintics were effective taenicides and some admitted that on occasions when they had purchased anthelmintics for dogs, they had not specified that they were required for the treatment of tapeworms. It was likely therefore that such anthelmintics as diethylcarbamazine citrate, piperzine phosphate and other preparations for round worms may have been used by some individuals.

The distribution of endemic coenurosis on farms where dogs received and did not receive anthelmintic treatment on 100 sheep rearing farms is set out in Table 3.

There was no significant difference in the prevalence of coenurosis on farms where dogs were treated regularly (47%) and where they were treated occasionally (51%). Similarly the difference in prevalence on farms where regular treatment was administered (47%) and farms where no treatment was administered (74%) was not significant ($X^2 = 2.929$ $p > 0.05$) nor was there any significant

difference in occurrence on farms where occasional treatment was carried out (51%) and on those where no treatment was given (74%) ($X^2 = 3.275$ $p > 0.05$).

The data on the feeding of raw meat, offal and heads and anthelmintic treatment was combined and related to the occurrence of coenurosis in sheep was the distribution of farms where coenurosis did and did not occur is set out in Table 4.

An examination of the data in the table revealed that there was no significant difference in the prevalence of coenurosis on farms where dogs consumed raw meat, offal and heads when they were treated regularly 4/11 (36.4) or occasionally 15/27 (55.5%). However there was a significant difference in prevalence on farms where dogs were treated regularly 4/11 ($X^2 = 10.9396$ $p < 0.001$) and occasionally 15/27 ($X^2 = 9.4696$ $p < 0.01$) when compared with those where no treatment was administered 34/38 (89%). The data for the 24 farms where dogs did not consume raw meat, offal and heads could not be subjected to such a statistical analysis because of the low numbers involved.

DISCUSSION

The feeding and management of dogs on the 168 farms were regarded as typical of that on sheep farms in Dyfed. The diet on most farms was considered to be barely adequate, in others unsatisfactory and was often reflected in the fairly poor body condition of the dogs. Cook (1965) and Cook and Clarkson (1971) collected information on the feeding of farm dogs in a number of areas in Great Britain and their findings were similar to those found in the present study. Indeed Cook (1965) reported that dogs on farms in Powys were often expected to fend for themselves for at least a part of the year which they did through scavenging.

Few farmers appreciated the need for dogs to be maintained on an adequate and balanced diet. Indeed few appreciated the basic requirements for a balanced diet, the majority believing that the dietary needs of a dog could be met by the feeding of flaked maize, maize meal or oatmeal porridge, supplemented by household scraps. Although most dogs consumed raw meat, offal and heads, such items were restricted to periods when sheep were slaughtered for human consumption or when sheep died. A proportion of farmers and shepherds believed that the regular feeding of animal protein influenced the performance of dogs, inducing lethargy or laziness and led to sheep worrying, although they were unable to produce evidence to this

effect. The tradition was to maintain dogs in a lean and fit condition, but sadly many did not come up to that standard and could be classified as thin and unfit.

From an epizootiological point of view, one of the most significant and indeed unsatisfactory practices was the feeding of raw meat, offal and heads, from healthy sheep slaughtered for human consumption. The fact that some of the slaughtered sheep were affected with coenurosis was not considered to be of any concern by many. The feeding of the same items from sheep dying on the farm was also unsatisfactory. Cook (1965) and Cook and Clarkson (1971) also found such practices in Wales and other areas of Great Britain. Stallbaumer (1985) reported that on 30/51 farms in Clwyd, sheep heads and offal were regularly obtained from abattoirs and fed to dogs and on two of these farms dogs were infected with T. multiceps. In the survey of cestode infection in dogs on the 100 sheep breeding and 18 wintering farms, dogs on 20 farms harboured T. multiceps (Chapter 3.4) and 14 of the owners subsequently admitted that sheep heads had been deliberately fed to dogs and two of them also admitted that the heads of "giddy" sheep were routinely fed.

In addition to the deliberate feeding of uncooked meat, offal and heads, dogs on 103/118 farms had opportunities to stray and gain access to sheep farms, thus providing an

additional dimension to the possible consumption of material containing cestode larvae. The danger of dogs becoming infected with T. multiceps through scavenging is rather remote as heads are only rarely removed from a carcase. Nevertheless straying and scavenging may lead to dogs becoming infected with other cestodes which could then be transmitted to farm animals and man. Despite assurances that dogs did not scavenge or indeed stray, it is likely that both occurred more frequently than farmers admitted. Stallbaumer (1985) found that although 70% of farmers claimed that their dogs did not stray, dogs from 8/35 (23%) farms in this group were found to be infected with cestodes which could only have been acquired through straying and scavenging.

There was a surprising degree of unawareness amongst the farmers of the consequences of the deliberate feeding of uncooked meat, offal and heads. This was evident in both sheep farmers and dairy farmers, although the latter were rather more ignorant of such matters. Whilst many knew that cysts in livers, lungs and omentum were in some way associated with tapeworms, they were not familiar with the life history of canine cestodes. Although farmers generally were aware that coenurosis was caused by a tapeworm, few realised that the feeding of a head from an affected sheep could infect dogs and then in turn sheep or cattle.

The data collected from the two groups of farmers clearly indicated that there was a link between the feeding of raw meat, offal and heads and cestode infection in dogs (Chapter 3.4). However it was apparent that the deliberate feeding of heads from healthy sheep slaughtered for human consumption, from sheep affected with coenurosis slaughtered for human consumption and from sheep dying on the farms was the most significant factor.

The reluctance of farmers to purchase canned meat and biscuits or biscuit meal, despite their high quality and convenience value was largely due to the cost of such items. Even allowing for the poor economic climate farming faced in the mid 1970's, it was difficult to understand why cost alone prevented their utilisation by the farming community.

Stallbaumer (1985) found that 44% of farmers in Clwyd obtained cestodial drugs from veterinary surgeons and 24% used such drugs on three or more occasions each year. In the Cilwycwm and Rhandirmwyn area sheep farmers only occasionally consulted veterinary surgeons outside the lambing season. In contrast agricultural merchants and even some pharmacists regularly visited sheep farms and supplied them with anthelmintics, sheep dips and other products. Furthermore agricultural merchants regularly attended markets at Llandovery, Lampeter and elsewhere and

maintained their contacts with farmers. It was not surprising therefore that anthelmintics for dogs were often obtained from sources other than veterinary surgeons.

Dogs on only 17/118 (14%) of the farms were regularly treated at three - four monthly intervals and a further 32 (27%) treated occasionally. This was in contrast with farmers in Clwyd, 24% of whom treated their dogs regularly at three to four monthly intervals and 72% of them treated dogs at least once every year (Stallbaumer 1985). The low proportion of farmers who treated their dogs may have been due to the fact that only a minority of them regularly obtained anthelmintics from veterinary surgeons in contrast to Clwyd farmers.

The reasons given for not treating dogs were many and varied, but the main ones were attributable to a lack of understanding of the life cycle of T. multiceps and other canine cestodes and cost. Several believed that coenurosis was derived from the sheep cestode Monezia expansa and therefore no other host was involved. Others did not accept that coenurosis was derived from dogs and a number believed that only young dogs were infected with tapeworms. The cost of anthelmintics loomed large in the minds of others. Although they had used Cestarsol previously, they had been persuaded to change to

bunamidine hydrochloride and the cost of treating one dog had increased from 30 - 50 pence to 110 - 150 pence per dose, which was considered prohibitive. The cost was often compared with that of treating a sheep with anthelmintics, at 30 - 50 pence per dose.

Although there was no significant difference in the occurrence of coenurosis on farms where dogs were treated regularly or occasionally, there was a significant difference when these levels of occurrence were compared with that on farms where no treatment was carried out. However more precise information on the anthelmintics used and the frequency of use would have been helpful in assessing their value.

In summary the study of the feeding, management and anthelmintic treatment of dogs on the 118 farms demonstrated that these factors were important in the infection of dogs with various species of cestodes and their transmission to farm livestock and man. It also emphasised that the control and possible eradication of hydatidosis, cysticercosis and coenurosis could only be achieved by making the farming community aware of the life cycles of canine cestodes and the measures that should be taken for their control.

| Food item | Percentage of dog consuming food items | | |
|---|--|--------------|-------|
| | Regularly | Occasionally | Total |
| Flaked maize, maize meal or oatmeal porridge | 62 | 20 | 82 |
| Household scraps | 20 | 40 | 60 |
| Offal, meat or bones specially purchased | 1 | 5 | 6 |
| Milk | 20 | 35 | 55 |
| Dog biscuits or meal | 5 | 10 | 15 |
| Canned dog meat | 1 | 6 | 7 |
| Offal and meat from sheep slaughtered for human consumption | 10 | 27 | 37 |
| Offal and meat from sheep carcasses or allowed to scavenge on carcasses | 4 | 31 | 35 |

Table 1 Food items consumed by dogs on 118 farms in Dyfed

| | Number of farms with endemic coenurosis | Number of farms with no endemic coenurus | Total |
|--|---|--|-------|
| Uncooked meat, offal heads consumed by dogs | *53 | 23 | 76 |
| No cooked meat, offal and heads consumed by dogs | 10 | 14 | 24 |
| Total | 63 | 37 | 100 |

Table 2 Distribution of endemic coenurosis in 100 sheep breeding farms where dogs were fed and not fed uncooked meat, offal and sheep heads (* $X = 6.8093$ $p < 0.02$)

| | Number of farms with endemic coenurosis | Number of farms with no endemic coenurosis | Total |
|------------------------------|---|--|-------|
| Dogs treated regularly | 8 (47%) | 9 (53%) | 17 |
| Dogs treated occasionally | 15 (51%) | 14 (49%) | 29 |
| Dogs not treated | 40 (74%) | 14 (26%) | 54 |
| Total | 63 | 37 | 100 |

Table 3 Association between anthelmintic treatment of dogs and coenurosis in sheep on 100 sheep breeding farms

| Dietary items and frequency of anthelmintic treatment | Number of farms with endemic coenurosis (%) | Number of farms with no endemic coenurosis (%) | Total |
|--|---|--|-------|
| Regular treatment, raw meat, offal and heads consumed | 4 (36.4) | 7 (63.6) | 11 |
| Regular treatment, no raw meat, offal and heads consumed | 4 (67) | 2 (33) | 6 |
| Occasional treatment, raw meat, offal and heads consumed | 15 (55.5) | 12 (44.5) | 27 |
| Occasional treatment, no raw meat, offal heads consumed | 0 | 2 (100) | 2 |
| No treatment, raw meat, offal and heads consumed | 34 (89.4) | 4 (10.6) | 38 |
| No treatment, no raw meat, offal and heads consumed | 6 (37.5) | 10 (62.5) | 16 |
| Total | 63 (63) | 37 (37) | 100 |

Table 4 Distribution of endemic coenurosis on farms where dogs were subjected to differing anthelmintic treatments and consumed or did not consume raw meat, offal and heads

Fig 1 The upper Tywi valley just north of
Rhandirmwyn village



3.4 The cestodes of farm dogs

The most obvious source of all larval (tissue) cestode infections for sheep and indeed cattle, must be farm dogs, especially working dogs that are in daily contact with flocks and herds. In a study of the epizootiology of coenurosis, the determination of the prevalence of Taenia multiceps infection in farm dogs must be an essential part and to this end it was decided to examine dogs on a number of sheep rearing farms and on farms where sheep especially lambs were overwintered.

MATERIALS AND METHODS

The farms chosen for the survey were those from which data on the feeding and management of dogs were collected, namely Group A consisting of 100 sheep rearing farms and Group B 18 wintering farms. Dogs on 75 farms were examined during the period May to October 1973 and the remainder during the same period in 1974.

Up to 10 farms were visited on any one day. Owners had been notified of the proposed visit about seven days previously and were requested to feed their dogs if possible before 18.00 hrs and not later than 20.00 hrs on the evening prior to the visit and not to feed them on the morning of the visit. They were also requested to provide

suitable accommodation with an impervious floor where dogs could be confined or tethered until the voided faeces could be collected. Most of the dogs were tethered in barns, stables or cowsheds, although some dogs had to be tethered on concrete areas in farm yards.

Cestarsol (May and Baker Ltd Dagenham) was administered to the dogs. It is a compound of 4-hydroxy-3-acetylamine-3 carboxylic acid methylester, which is a white powder, odourless, tasteless and readily soluble in water. The commercial preparation consists of 18 mg tablets and the recommended dose rate is 1 tablet per 15 lb (6.8 kg) body weight up to a maximum of four tablets.

The group of farms selected for the day were visited in sequence, the dogs weighed with a spring balance and the appropriate number of tablets administered. Because of the size of the tablets it was not possible to administer less than half a tablet, thus some dogs received rather more and others rather less than the recommended dose. The farms were re-visited between two and three hours later to assess the effectiveness of the treatment and to collect the voided faeces. A child's plastic spade and a metal spatula were used to collect the faeces which was then transferred into screw capped six or eight ounce wide mouthed plastic containers.

At the laboratory an equal volume of 10 per cent formalin was added to the faeces to give an estimated final concentration of 5 per cent. The faeces samples were then washed first through a metal sieve of 10 meshes to the square inch and the large mature cestodes removed with a forceps and then through another sieve of 100 meshes to the square inch. The residue was examined by the naked eye and then with the aid of a dissecting microscope for the presence of cestodes. Those recovered were stored in 5 or 10 per cent formol saline until they could be identified.

Each parasite was examined macroscopically and microscopically and when necessary strobila fragments stained with 3 per cent Mayer's haemalum or haematoxylin-eosin. Scolices were mounted "face on" in lactophenol, and the rostellum, suckers, large and small hooks examined and measured. The identity of Taenia species was based on criteria listed by Verster (1969), D. caninum on the the description by Soulsby (1968) and E. granulosus on the description of Sweatman and Williams (1963). A selection of identified cestodes were examined and their identity confirmed by Professor Gwendolen Rees, University College of Wales, Aberystwyth, a former student of Professor Rees, Mrs V Jones, Carmarthen, Mr S Prudhoe of the British Museum (Natural History) and members of the

Parasitology Department of the Central Veterinary Laboratory of MAFF, and these were then used for reference purposes.

RESULTS

Group A The results of the survey of dogs on sheep rearing farms have been published elsewhere (Williams 1976a) but are now presented in greater detail.

Of the 383 dogs on the 100 sheep rearing farms only 357 were treated with Cestarsol. The remainder were excluded because of pregnancy, age, chronic illness, eg arthritis, nephritis, neoplasia, or because owners requested that they should not be treated. Twenty six were of the terrier type or crossbrds, the remainder were sheepdogs of the Border or Welsh Collie breeds. Three hundred and twenty (89.6%) of those treated were successfully purged and 209 (65%) were males and 111 (35%) females. A number of dogs were between nine and 12 months of age and these are included in the total aged one year. Similarly dogs over 10 years of age are included in the 10 year and over age group. It should be emphasised however that it was difficult to obtain the precise age of some dogs, especially older dogs, the owners having to relate the

birth or purchase to a particular event such as a wedding, funeral, farm sale or agricultural event. The age distribution of male and female dogs is shown in Fig 1.

A total of 202 (63%) male and female dogs on 76 farms were infected with one or more species of cestode and of these 137 (67%) were male and 65 (33%) were female. The proportion of infected dogs in each age group is demonstrated in Fig 2 and the worm burden of an infected dog is illustrated in Fig 3(a).

There was no significant difference ($X^2 = 1.3$ $p > 0.10$) between the proportion of males infected 137/209 (65.6%) and females infected, 65/111 (58.6%). Nor was there any significant difference in the age distribution of infected male and female animals ($X^2 = 7.91$, 9 df $p > 0.10$). Similarly there was no significant difference between the age distribution of infected and non infected dogs. Infection in dogs of six years of age and over, 69/107 (64.5%) was marginally higher than those under six years of age, 133/213 (62.4%) but again the difference was not significant ($X^2 = 0.06$).

The prevalence of the cestode species recovered from the dogs and the number of farms on which dogs were infected with each species are set out in Table 1.

The commonest species of cestode indentified was T. hydatigena with 147 (46%) dogs infected on 72 farms. E. granulosus was found in 48 (15%) of dogs on 17 farms and 37 (11.5%) dogs on 20 farms were infected with T. multiceps. Only 22 (7.0%) dogs on 7 farms were infected with T. ovis. Of the cestodes with larval stages in hosts other than farm livestock T. pisiformis was the commonest with 45 (14%) dogs on 23 farms infected. T. serialis was recovered from 14 (4%) dogs on 3 farms, and D. caninum from 26 (8%) dogs on 12 farms.

The numbers of dogs that were infected with cestodes were generally too low to subdivide into individual age groups. Therefore to obtain a normal distribution and carry out tests of significance, counts for male and female dogs over and under six years old this were transformed to their square roots for all cestode species except E. granulosus, where because of the higher number of worms, a logarithmic transformation was used. Reconverted mean values for male and female dogs of less than six or more than six years old are set out in Table 2. Where numbers were sufficient an analysis of variance was performed on the transformed variables to determine the significances of differences between groups of which however there were none (Table 2).

The distribution of worm burdens of individual cestode species in infected dogs is demonstrated in Table 3.

The majority of dogs were infected with more than one species of cestode and as expected the heaviest burdens were those of E. granulosus, one dog yielding 3,200 specimens and another 500 of this species and 159 specimens of T. multiceps. The mean burden per infected dog of E. granulosus was 30, much higher than the mean burden of other cestode species.

The mean burden of T. multiceps was 6.7 although one five year old dog yielded 159 worms. The mean burden of T. serialis, a cestode which also has a coenurus as the larval stage was 8.1. The majority of dogs infected with these species had burdens of less than 10 parasites.

The distribution of the worm burdens of T. ovis, T. pisiformis and D. caninum was similar with mean burdens of 2.8, 4.0 and 4.7 respectively. Most dogs had burdens of less than six parasites.

Taenia hydatigena was the most prevalent of the seven species of cestode. The mean burden was 2.1, but 92/147 (62%) dogs had burdens of one or two worms only.

Group B farms The results of the survey of dogs on wintering farms have been published (Williams 1976a), but are now presented in greater detail.

All 39 dogs on the 18 farms were of the Border or Welsh Collie breeds and all were treated with Cestamol. Thirty (76.9%) of these were purged compared with 320/357 (89.6%) on the sheep rearing farms which was significantly less ($X^2 = 4.37$, $p < 0.05$). Twenty three (76.6%) of the purged dogs were male. Cestodes were recovered from 19/30 (63.3%) dogs on 8 farms and of these 14 (73.7%) were male and 5 (26.3%) female.

The cestode infection rate in males, 14/23 (61%) was not significantly lower ($X^2 = 0.004$ $p > 0.5$) than that in females 5/7 (71%), but it must be emphasised that only a relatively small number of dogs were infected. Similarly the number of infected dogs less than six years of age, 16/24 (67%) did not differ significantly ($X^2 = 0.08$ $p > 0.5$) from the number over six years of age that were infected 3/6 (50%). Nor was there any significant differences in the age distribution of infected and non infected dogs ($X^2 = 3.69$ $df\ 7$ $p > 0.5$) or in the age distribution of male and female infected dogs ($X^2 = 8.00$ $p > 0.5$). The age distribution of infected and non infected male and female dogs is set out in Table 4.

The prevalence of cestode species in the dogs and the number of farms with infected dogs are set out in Table 5. Most dogs were infected with one or more species of cestode. As for dogs on the sheep rearing farms, T. hydatigena was the commonest species identified with 7/30 (23%) of dogs infected on five farms. Only two dogs (7%) on one farm were infected with E. granulosus. T. multiceps was recovered from three dogs (9.6%) on two farms. No specimens of T. ovis were recovered. Of the other three species of cestode recovered, D. caninum was the commonest with 14/30 (47%) of dogs infected on six farms. Two dogs (7%) on two farms were infected with T. serialis and another five (17%) dogs on four farms were infected with T. pisiformis.

Because of the relatively small numbers of dogs infected with each cestode species it was not possible to carry out a comprehensive analysis as was done for dogs on the sheep rearing farms. Therefore only the distribution of the worm burdens of each species of cestode in the infected dogs is presented in Table 6.

DISCUSSION

Arecoline hydrobromide has for many years been used for the removal of tapeworms from the intestinal tract of dogs. It acts directly on these parasites, causing them

to relax and to become detached from the intestinal mucosa. In addition to this it also produces an increased peristalsis of the small intestine, which eliminates the relaxed cestodes, usually within two hours of administration. These properties thus make it an ideal agent for use in a survey of tapeworm infection in dogs.

The only preparation available in Great Britain was arecoline - acetarsol, although the efficiency of arecoline allegedly is not improved by combination with acetarsol. The product was marketed by May and Baker Ltd, Dagenham (now RMB Animal Health Ltd, Dagenham), under the trade name of Cestarsol, but soon after the survey commenced the product was withdrawn by the company. However they kindly agreed to make sufficient supplies available for completion of the survey of farm, pet and hunting dogs.

The efficiency of arecoline against E. granulosus is 25 - 60% (WHO Bulletin 1960) although Coles (1983) claims a greater efficiency against this parasite. In contrast it is widely recognised that arecoline is highly efficient in eliminating the larger cestodes (taeniids) from the intestinal tract of dogs. In preliminary trials at the laboratory it was demonstrated at post mortem examination that experimental infections of T. multiceps were completely eliminated by treatment with Cestarsol at the

recommended dose rate. The results obtained from this survey of farm dogs, and from the surveys of hunting dogs and pet dogs therefore must have reflected fairly accurately the prevalence of cestodes, especially taeniids at that time.

Most of the larger sheep rearing farms had three or more dogs and it was surprising that 37/320 (11.5%) of the dogs purged were 10 years of age or over. The majority of working dogs are retired at eight or nine years of age, mainly because of deteriorating eye sight and hearing. However some dogs of nine, ten years or older may still be used for shepherding, although they may be confined to working on sheep pastures near the farmstead. Farmers were reluctant to destroy retired working dogs, unless it became necessary on health grounds, arguing that a sheep dog that has given valuable service over a period of years, deserved to live as long as possible.

Sheep farmers generally prefer male to female dogs, because of the problems that occur twice each year with the onset of heat and unplanned pregnancies. Nevertheless if a good working dog became available, it would be purchased and the complications from periodic heat periods overcome by either retaining bitches only on the farm or

by seeking veterinary treatment to suppress heat. At the time of the survey 111/320 (35%) bitches were on the farms.

The overall efficiency of purgation recorded in the survey was similar to that recorded by others, notably Walters (1977). However it is difficult to account for the difference in purgation rate in the dogs on the two groups of farms; but two factors may have been responsible. At least two of the dogs on the wintering farms had been inadvertently fed on the day they were treated and four of the dogs on these farms were over 60 lbs body weight and therefore in comparison with lighter dogs were underdosed.

In the survey 202/320 (63%) of dogs on 76 (76%) sheep rearing farms were infected with one or more species of cestode. This prevalence was similar to that found in Powys by Cook (1965) who found 71% of dogs on 70% of farms infected with cestodes. Walters (1977) however reported that there had been a significant reduction in the numbers of dogs infected with cestodes in Powys, which was reflected by his findings that only 13 - 20% of dogs on 30 - 40% of the farms he visited on three occasions were infected. This dramatic reduction in prevalence can be

readily explained by the increased publicity given to human hydatid disease in the area which had led to preventive action being taken.

The high prevalence of cestode infection in dogs in the Cilwch and Rhandirmwyn area contrasted with the very low prevalence found by Edwards, Hackett and Herbert (1979a) and Stallbaumer (1985) in farm dogs in North Wales. Why this should be is not entirely clear but a number of factors may be responsible for this difference. Such factors include the number, feeding and management of dogs, accessibility of sheep grazings to dogs, grazing management and prevalence of tissue cestodes in sheep.

Information on the management, feeding and anthelmintic treatment was collected at the time of the visits and information on carcase disposal had been provided by the owners when completing the questionnaire. This information was correlated with the presence of, and species of cestodes in the farm dogs. The cestodes were grouped into those with intermediate stages in sheep and those with intermediate hosts other than sheep. The data are set out in Table 7.

On 92/118 sheep rearing and wintering farms dogs consumed meat and offal including heads from sheep killed for human consumption or from carcasses of dead sheep and 170/240 were infected with cestodes. Cestodes with intermediate

stages in sheep were demonstrated in 141/170 (83%). In addition, 29/170 (17%) were infected with either T.serialis, T.pisiformis or D. caninum with intermediate stages not in sheep. On 26 farms owners claimed that dogs did not consume meat and offal, including heads from sheep but 51/110 (46%) dogs were infected with cestodes, and only 13/54 (25%) were infected with the four cestodes that have intermediate stages in sheep. Nevertheless the claimed difference in feeding methods resulted in a highly significant ($X = 62.2$ $p < 0.001$) difference in the prevalence of cestodes using the sheep as intermediate host (Table 7). Even so despite the assurances given by the 26 farmers that their dogs did not consume sheep meat and offal and that they did not scavenge, it was obvious that raw meat had been consumed. Thus the high prevalence of cestodes can in part be explained by the standard of feeding and management of the dogs. Stallbaumer (1985) reported that in North Wales that 23.4% of dogs on farms where dogs had access to sheep meat were infected with cestodes, but only 4.6% of dogs on farms where dogs had no access to such items.

Stallbaumer (1985) drew attention to the high number of dogs in Powys (12000 - 16000) compared to Clwyd (5000 - 6700) and regarded this as an important factor in the epizootiology of cestode infection in dogs and farm livestock. Whilst there was no precise information

available on the number of dogs in Dyfed but is likely to have been far more than the total for Powys in view of the fact that on the 552 farms covered by the postal survey these were probably in excess of 2000 dogs.

Dogs were reported to be regularly treated with taeniocides on 17/118 (27%) farms and occasionally treated on 32/118 (27%). Anthelmintic treatment of dogs is related with the canine cestode infections on these farms in Table 8.

The relationship between anthelmintic treatment and cestode infection is highly significant ($X^2 = 63.7136$, $p < 0.001$) but it must be emphasised that the dogs were only purged for this survey on a single occasion and close monitoring may have produced a different picture. Furthermore it was not possible to identify the anthelmintic used on many farms because either owners did not know the product that they had obtained from pharmacists, travelling salesmen or veterinary surgeons or they had none that could be readily identified. Nevertheless the difference in prevalence of cestodes in dogs on farms where dogs were treated regularly compared with those on farms where dogs were treated occasionally was significant ($X^2 = 7.35$ $p < 0.01$). There was also a highly significant difference between the prevalence of

cestodes in dogs on farms where occasional treatment was given compared with that in dogs where no treatment was given ($X^2 = 16,8$ $p < 0.001$).

The combined data on feeding of uncooked meat and offal (including heads) and anthelmintic treatment is related to canine cestode infection in Table 9.

On those farms where uncooked meat and offal were fed to the dogs, there is no significant difference ($X^2 = 0.15$ $p > 0.10$) in infection between dogs with occasional and regular treatment with anthelmintic drugs but there is ($X^2 = 4.8$ $p < 0.05$) between treated and untreated dogs (Table 9).

The numbers of farms, where it was claimed that no uncooked meat and offal were consumed by dogs, are too few for statistical analysis (Table 9).

In addition to the deliberate feeding of sheep meat and offal and anthelmintic treatment of dogs another factor which had to be considered was the accessibility of sheep grazings to dogs. The fact that dogs, especially male dogs stray from farmsteads, provide opportunities for scavenging. As indicated in Chapter 3.3 few dogs were confined or tethered during the day. Mortality in adult sheep is highest during the winter and spring when ewes

are pregnant or newly lambed. For most of this period flocks are maintained on pastures near the farmstead, within easy reach of straying dogs, and the carcasses of dead sheep would be easily available to them. This undoubtedly accounted for cestode infection in dogs on farms where owners and shepherds insisted that their dogs did not consume uncooked sheep meat and offal.

The high prevalence of cestodes in dogs in South and mid Wales compared with dogs in North Wales noted by Edwards, Hackett and Herbert (1979a) and Stallbaumer (1985), is reflected in the high incidence of larval cestodes in sheep and cattle in Dyfed. Over a period of 19 years, the author has consistently recorded an incidence of approximately 30 per cent of Cysticercus tenuicollis (larval stage of T. hydatigena) infection in sheep over six months of age subjected to post mortem examination at the Aberystwyth and Carmarthen Veterinary Investigation Centres. Similarly carcasses of aged ewes examined at these laboratories revealed an incidence of hydatid infection of 15 - 20 per cent and periodic surveys of cast ewes, those drafted from flocks because of tooth loss or other reasons, at local abattoirs revealed that approximately 25% were affected with hydatidosis. During the compulsory slaughter of cattle under the Brucellosis Eradication Schemes between 1972 and 1976, 25 - 30% adult beef cattle from certain areas in the north of the old

county of Carmarthen had hydatidosis. A much higher incidence was found in slaughtered cattle originating from herds in Powys in the same period, with up to 80% infection. It was not surprising therefore that 63% of dogs in sheep rearing farms were harbouring cestodes.

The lowest percentage infection by cestodes was found in one and nine year old dogs and the highest in three and eight year old dogs. Edwards, Hackett and Herbert (1979a) in a study of farm dogs as definitive hosts of taeniid cestodes found that infections were more or less evenly distributed in all age groups, but more prevalent in dogs of 2.5 - 3.5 years of age, and significantly less prevalent in dogs up to 1.5 years of age. The lower prevalence rate of cestodes in one year old dogs, found by Edwards, Hackett and Herbert (1979a) and in the present survey may be due to the fact that such dogs are kept under much closer control and still undergoing training. Again the higher infection rate found in three year old dogs was also confirmed by Edwards, Hackett and Herbert (1979a). It was difficult to explain why the nine year old dogs had a lower infection rate, but it may have been partly due to the small number of dogs in this age group and to their either not being fed significant amounts of uncooked meat and offal or that because of old age were not scavenging. It was obvious from this survey and that by Edwards, Hackett and Herbert (1979a) that dogs of all

ages acquire cestode infections and that immunity to cestodes in dogs is variable and not long lasting as reported by Herd (1970) and Gemell (1983). It was also interesting to note that the burdens of individual cestodes were not significantly different in dogs under six years of age and those over six years of age, nor was there any significant difference in the burdens of male and female dogs.

Echinococcus granulosus was found in a total of 48 (15%) dogs on 17/100 sheep rearing farms and 2 (7%) dogs on one of the wintering farms. This compared with 23% of dogs examined by Cook (1965) in mid Wales and 17.8% of those examined by Walters (1977) in the same area. The hydatid cysts, usually located in the liver and lungs are readily accessible to scavenging dogs and farmers regularly feed these organs from slaughtered sheep to their dogs.

Taenia multiceps was recovered from 37 (11.5%) of dogs on 20/100 sheep rearing farms and from 3 (9.6%) dogs on 2/18 wintering farms. The relationship between infections in dogs and the occurrence of coenurosis in sheep on these farms was interesting and is set out in Table 10.

On the 72 sheep rearing farms with a recent history of coenurosis 36/255 (14.1%) of dogs on 19 farms were infected with T. multiceps. This was significantly higher

($X^2 = 6.83$ $p < 0.01$) than the prevalence of 1/65 (1.5%) in dogs on the remaining 28 farms, but did not differ significantly from the prevalence 3/30 (10%) on the wintering farms ($X^2 = 0.12$ $p > 0.05$). Cook (1965) found 8% of dogs in Powys infected with the cestode. Edwards, Hackett and Herbert (1979a) and Stallbaumer (1985) found 3.5% of dogs in north Wales infected with T. multiceps.

On the two wintering farms with infected dogs (Table 10) the owners admitted that dogs had been fed on the carcasses and heads of two "giddy" sheep which had been slaughtered on the farm by the owners of the overwintered sheep and on the head and carcass of one sheep that had died, probably from coenurosis. On one of these farms two calves 3 - 4 months of age developed coenurosis shortly after the dogs had been purged. These calves had not been out of doors. The two dogs on the farm were housed at night in a section of the calf house where hay bales for the calves were stored, but they were prevented from having direct contact with the calves. There was strong circumstantial evidence that the dogs had deposited proglottids and oncospheres of T. multiceps on the hay bales, which had subsequently infected the calves.

The owners of dogs on the sheep rearing farms also admitted that they sometimes allowed dogs to feed on sheep heads including the heads of "giddy" sheep. However the

owner of the single infected dog on the single farm without a recent history of coenurosis (Table 10) was adamant that it had not been allowed access to sheep heads.

When farm dogs scavenge on carcasses, they attack the abdomen initially and then gain access to the thoracic organs through the diaphragm. Other scavengers, birds, foxes and stray dogs also feed on the carcass, but seldom is the head removed from the carcass. It was difficult therefore to see how dogs became infected unless they were deliberately fed with the heads of sheep, a view which is shared by Stallbaumer (1985).

The high prevalence of T. hydatigena in dogs on both groups of farms reflected the high prevalence of the larva of this cestode in the intermediate hosts, especially sheep. It was not surprising to find this prevalence as the cestode larvae were readily accessible through scavenging on carcasses and from the liver and omentum of sheep slaughtered for human consumption. Cook (1965) recorded a prevalence of 55.2% in dogs in Powys and Hackett and Walters (1979) reported a prevalence of 26.4% in the same area. The low rate of cestode infection in dogs in north Wales in comparison is reflected in a prevalence of 11.35% in farm dogs in Snowdonia. The prevalence of 46% in dogs on sheep rearing farms and 22.6%

in wintering farms recorded in this survey indicated clearly that farm dogs were readily gaining access to infected material.

The overall low prevalence of T. ovis in comparison confirmed that the larva Cysticercus ovis, was relatively uncommon in sheep in Dyfed. Other workers Cook (1965), Hackett and Walters (1980b), Edwards, Hackett and Herbert (1979a) recorded a prevalence of 1.4 - 3.5% in dogs in mid and north Wales, which compares with 6.3% in the present survey.

The other species of cestodes identified were of little importance in the context of animal and human health: T. serialis was recovered from 4.6% of dogs and is worthy of mention because of its similarity to T. multiceps. Cook (1965) found this cestode in 2.3% of dogs and Hackett and Walters (1979) in 0.8%. T. pisiformis was far more common and the overall prevalence of 14.2% compared with 6.6 - 10.3% in dogs recorded by Cook (1965), Hackett and Walters (1980b) and Edwards, Hackett and Herbert (1979a).

The survey of cestodes in farm dogs together with the information gathered on their feeding and management, clearly demonstrated a relationship between the prevalence of certain species of cestode and the consumption of uncooked meat and offal and anthelmintic treatment of

dogs. Furthermore a firm link was established between the presence of T. multiceps in the dogs and the feeding of sheep heads. Although a further survey or surveys would have been desirable valuable information on the epizootiology of coenurosis was obtained.

| Species of cestode | Number of infected dogs (%) | Number of infected farms |
|------------------------|-----------------------------|--------------------------|
| <u>E. granulosus</u> * | 48 (15) | 17 |
| <u>T. multiceps</u> * | 37 (11.5) | 20 |
| <u>T. ovis</u> * | 22 (70) | 7 |
| <u>T. hydatigena</u> * | 147 (46) | 72 |
| <u>T. serialis</u> ° | 14 (4) | 3 |
| <u>T. pisiformis</u> ° | 45 (14) | 23 |
| <u>D. caninum</u> ° | 26 (8) | 12 |

* Intermediate stages in sheep

° Intermediate stages not in sheep

Table 1 Prevalence of cestodes in dogs on 100 sheep rearing farms and the number of farms with dogs infected with each cestode species

| Species of cestode | Mean cestode counts Male dogs | | Mean cestode counts Female dogs | | Overall mean counts | RSD |
|----------------------|----------------------------------|----------|------------------------------------|---------|---------------------|------|
| | < 6yrs | > 6yrs | < 6yrs | > 6yrs | | |
| <u>E.granulosus</u> | 26(14) | 26(16) | 33(11) | 64(3)* | 30(47) | 0.68 |
| <u>T.multiceps</u> | 6.4(17) | 8.3(5)* | 7.2(10) | 5.0(4) | 6.7(36) | 0.82 |
| <u>T. ovis</u> | 2.9(7) | 1.7(5) | 3.8(8) | 2.2(3) | 2.8(23) | - |
| <u>T. hydatigena</u> | 2.1(6) | 2.0(37)* | 2.1(32) | 2.3(14) | 2.1(146) | 0.42 |
| <u>T. serialis</u> | 9.3(6) | 5.0(4) | 7.5(2) | 12.3(2) | 8.1(14) | |
| <u>T. pisiformis</u> | 4.2(19) | 4.0(11) | 3.8(14) | 5.0(1) | 4.0(45) | 0.26 |
| <u>D. caninum</u> | 4.4(13) | 4.3(13) | 5.4(9) | 5.0(1) | 4.7(26) | |

RSD: Residual standard deviation of transformed values

* Omitting one outlying value () number of dogs

Table 2 Mean cestode counts for 7 cestode species in male and female dogs on 100 sheep rearing farms

| | No of dogs negative | Number of dogs 2-10 | with worm burdens of 11-50 | 51-100 | 101-200 | 300-600 | 3600* | Mn** brdn |
|-----------------|------------------------|------------------------|-------------------------------|--------|---------|---------|-------|--------------|
| <u>E. gran.</u> | 272 | 15 | 10 | 10 | 2 | 2 | 1* | 30 |

| | No of dogs negative | No of dogs 1-5 | with worm burdens of 6-10 | 11-20 | 21-30 | 159* | Mean burden |
|---------------------|------------------------|-------------------|------------------------------|-------|-------|------|----------------|
| <u>T. multiceps</u> | 283 | 19 | 11 | 2 | 4 | 1* | 6.7 |
| <u>T. serialis</u> | 306 | 6 | 5 | 2 | 1 | 0 | 8.1 |

| | No of dogs negative | No of dogs 1-2 | with worm burdens of 3-4 | 5-6 | 7-8 | 9-10 | Mean burden |
|----------------------|------------------------|-------------------|-----------------------------|-----|-----|------|----------------|
| <u>T. ovis</u> | 297 | 6 | 16 | 0 | 0 | 1 | 2.8 |
| <u>T. pisiformis</u> | 275 | 1 | 35 | 8 | 1 | 0 | 4.0 |
| <u>D. caninum</u> | 294 | 0 | 11 | 13 | 2 | 0 | 4.7 |

| | No of dogs negative | No of dogs 1 | with worm burdens of 2 | 3 | 4 | 5 | 6-10 | 37* | Mean burden |
|----------------------|------------------------|-----------------|---------------------------|----|----|---|------|-----|----------------|
| <u>T. hydatigena</u> | 48 | 48 | 44 | 31 | 17 | 4 | 2 | 1 | 2.1 |

* Omitted from statistical analysis

** Mean per infected dog

Table 3 The frequency distribution of worm burdens of individual cestode species in dogs on 100 sheep rearing farms

| Age (yrs) | Male dogs | | | Female dogs | | |
|--------------|-----------|--------------|-------|-------------|--------------|-------|
| | Inftd(%) | Non inftd(%) | Total | Inftd(%) | Non inftd(%) | Total |
| 1 | 3(60) | 2(40) | 5 | | | |
| 2 | 1(33) | 2(66) | 3 | 2(100) | 0 | 2 |
| 3 | 4(57) | 3(43) | 7 | 1(100) | 0 | 1 |
| 4 | 2(100) | 0 | 2 | 0 | 1(100) | 1 |
| 5 | 2(100) | 0 | 2 | 1(100) | 0 | 1 |
| 6 | | | | 1(50) | 1(50) | 2 |
| 7 | 1(100) | 0 | 1 | | | |
| 8 | | | | | | |
| 9 | 1(33) | 2(66) | 3 | | | |
| Total | 14(61) | 9(39) | 23 | 5(71) | 2(29) | 7 |

Table 4 Age distribution of cestode infected and non infected male and female dogs on 18 wintering farms

| Species of cestode | Number of infected dogs (%) | Number of infected farms |
|----------------------|-----------------------------|--------------------------|
| <u>E. granulosus</u> | 2 (7) | 1 |
| <u>T. multiceps</u> | 3 (10) | 2 |
| <u>T. ovis</u> | 0 | 0 |
| <u>T. hydatigena</u> | 7 (23) | 5 |
| <u>T. serialis</u> | 2 (7) | 2 |
| <u>T. pisiformis</u> | 5 (17) | 4 |
| <u>D. caninum</u> | 14 (47) | 6 |

Table 5 The prevalence of cestodes in dogs on 18 wintering farms

| Cestode species | No of dogs negative | No of dogs with worm burdens of | | | | | | | | | |
|----------------------|------------------------|---------------------------------|---|---|---|---|---|----|----|----|----|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 10 | 11 | 16 | 21 |
| <u>E. granulosus</u> | 28 | | | | | | | 1 | | | 1 |
| <u>T. multiceps</u> | 27 | | | | | 2 | | | 1 | | |
| <u>T. hydatigena</u> | 23 | 1 | 2 | | 2 | 1 | 1 | | | | |
| <u>T. serialis</u> | 28 | | | | 1 | | | | | 1 | |
| <u>T. pisiformis</u> | 25 | 1 | | 1 | 1 | 1 | | | | | |
| <u>D. caninum</u> | 16 | | | 1 | 1 | 3 | 2 | | | | |

Table 6 Frequency distribution of cestode species worm burdens in infected dogs on 18 wintering farms

| | No of farms | No of dogs purged with | No of dogs infected with cestodes(%) | No of dogs with cest- odes with inter- mediate stages in Sheep(%) Not in sheep | |
|--|----------------|------------------------------|---|---|--------|
| Raw sheep meat 92 offal consumed or carcasses access (a) | 92 | 240 | 170(71) | 141(83)* | 29(17) |
| No sheep meat 26 offal nor carcasses access (b) | 26 | 110 | 58(46) | 13(25) | 38(75) |
| Total | 118 | 250 | 221 | 154 | 67 |

* (a) versus (b) $X^2 = 62.2$ $p < 0.001$

Table 7 Relationship between the consumption of sheep meat and offal and type of cestodes in 221 farm dogs on 118 sheep rearing and wintering farms

| Frequency of anthelmintic treatment | No of farms | No of farms with infected dogs | Number of farms with no infected dogs |
|-------------------------------------|-------------|--------------------------------|---------------------------------------|
| Regular | 17 | 2 | 15 |
| Occasional | 32 | 18 | 14 |
| Never | 69 | 64 | 5 |
| Total | 118 | 84 | 34 |

$$\chi^2 = 63.7136, \text{ df } 2 \quad p < 0.001$$

Table 8 Relationship between anthelmintic treatment and cestode infections in dogs on 118 wintering and sheep rearing farms

| Feeding regime and anthelmintic treatment | No of farms with no infected dog (%) | No of farms with infected dogs (%) | Total |
|--|--------------------------------------|------------------------------------|-------|
| Consuming uncooked meat and treated regularly | 4 (40) | 6 (60) | 10 |
| Not consuming uncooked meat and treated regularly | 4 (57) | 3 (43) | 7 |
| Consuming uncooked meat and treated occasionally | 9 (41) | 13 (59) | 22 |
| Not consuming uncooked meat and treated occasionally | 1 (25) | 3 (75) | 4 |
| Consuming uncooked meat and not treated | 10 (17) | 50 (83) | 60 |
| Not consuming uncooked meat and not treated | 6 (40) | 9 (60) | 15 |
| Total | 34 (29) | 84 (71) | 118 |

Table 9 Relationship between anthelmintic treatment and consumption of uncooked meat with cestode infection in dogs on 118 sheep rearing and wintering farms

| | | Total no of dogs purged | No of dogs infected with <i>T.</i> <i>multiceps</i> (%) | No of infec- ted farms |
|--|-----|----------------------------|--|---------------------------|
| No of sheep rearing farms with recent history of coenurosis | 72 | 255 | 36 (14) | 19 |
| No of sheep rearing farms with no recent history of coenurosis | 28 | 65 | 1 (1.5) | 1 |
| No of wintering farms with recent history of coenurosis | 18 | 30 | 3 (10) | 2 |
| Total | 118 | 320 | 40 | 22 |

Table 10 Relationship between *T. multiceps* infection in dogs and the occurrence of coenurosis in sheep on 100 sheep rearing and 18 sheep wintering farms



Figure 1: Age distribution of male and female dogs on 100 sheep rearing farms.

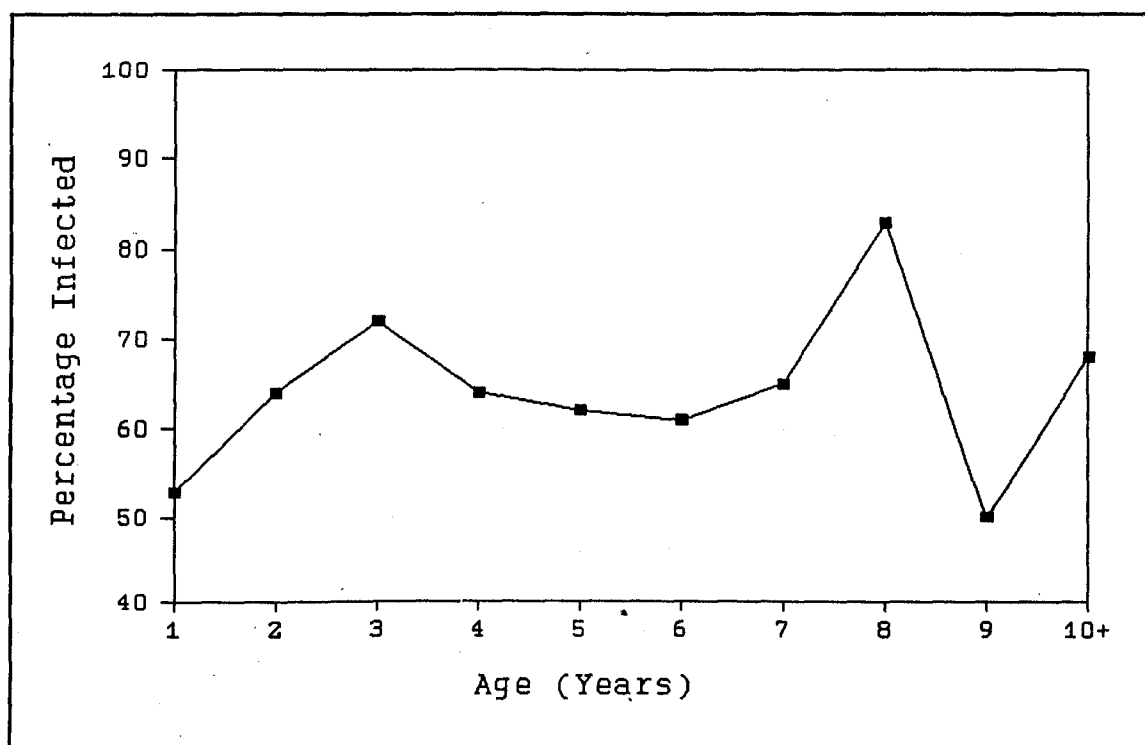


Figure 2: Percentage of cestode infected dogs in all age groups on 100 sheep rearing farms.

Fig 3 Cestode burdens of a farm dog,
hound and fox

a) The cestode burden of a five year old farm dog. Although the majority of the cestodes are mature, because of the "crowding effect", their lengths are much shorter than those found in lighter burdens (x 0.2)

b) The cestode burden in a hound. As in a) there is evidence of a "crowding effect" (x 0.2)

c) Cestodes recovered from a mature male fox. Individual cestodes are much shorter than those of the same species found in dogs and hounds (x 0.5)

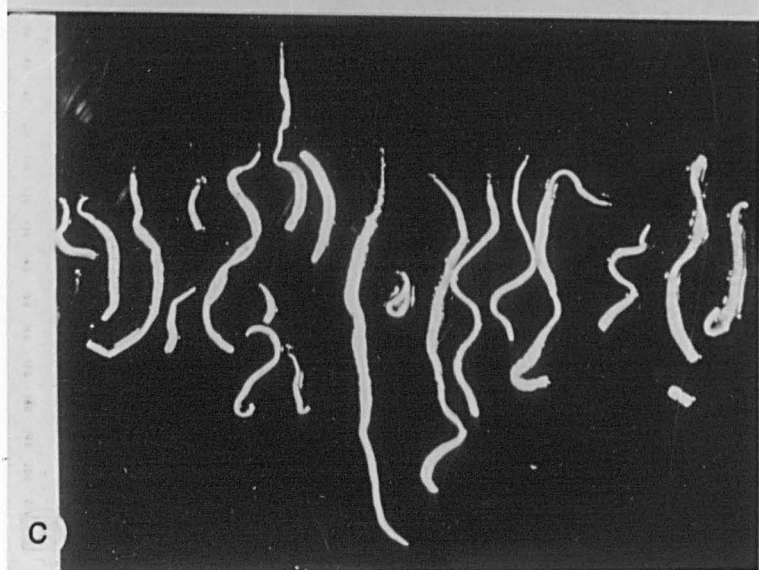
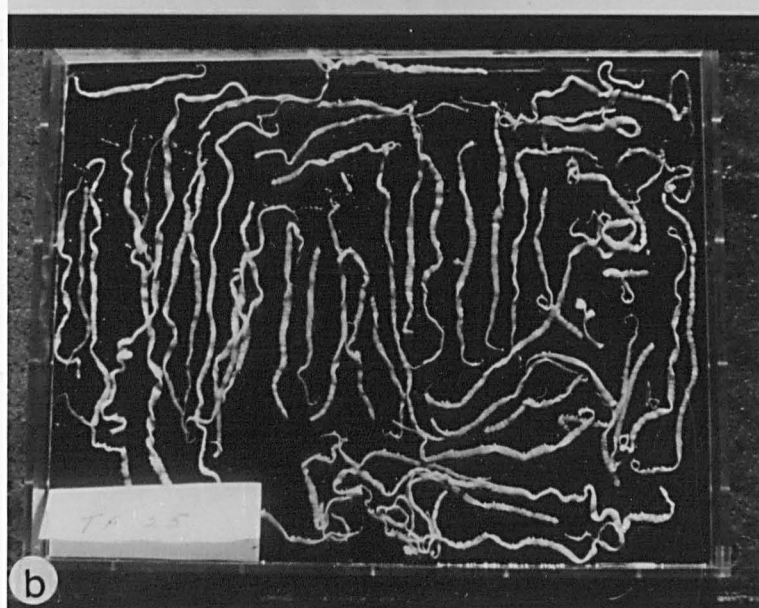
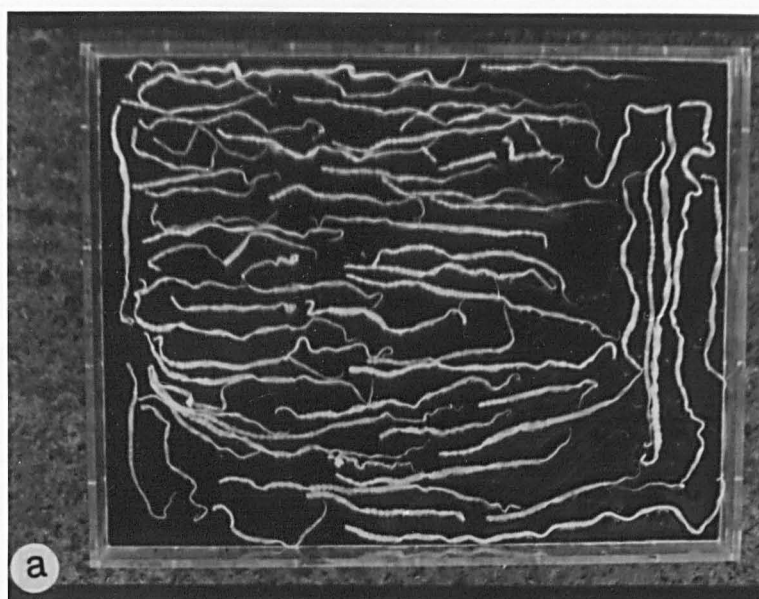
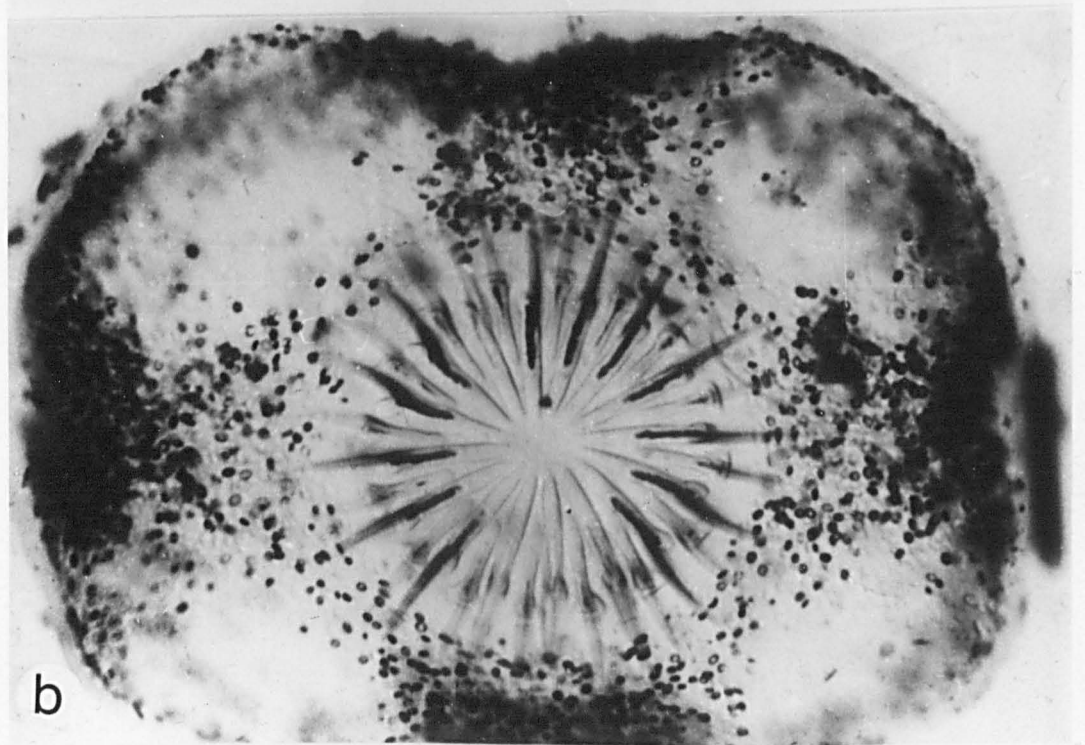
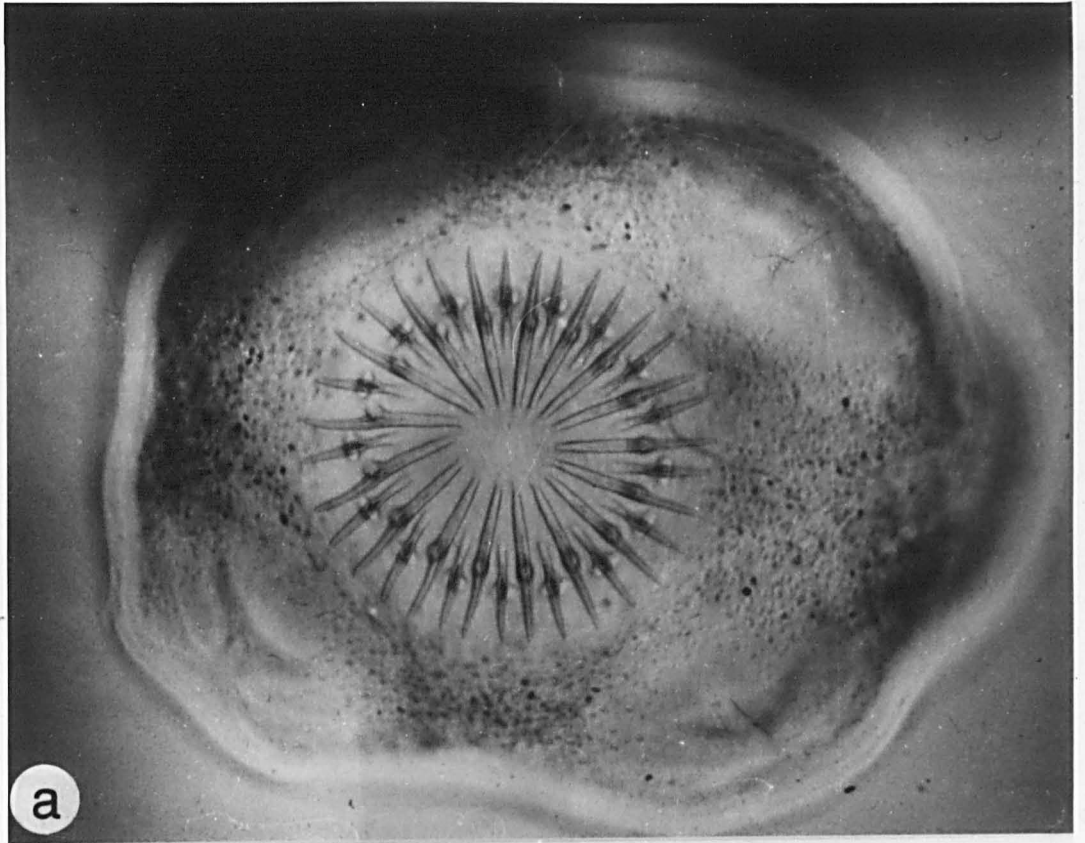


Fig. 4 Scolices of two species of adult cestodes recovered from farm dogs (x 20)

a) Taenia hydatigena (x 167)

b) Taenia pisiformis (x 167)



3.5 The cestodes of fox hounds and their relationships with diet and anthelmintic treatment

Several packs of hounds hunted over large areas of Dyfed and most consumed the carcasses and offal of cattle and sheep which died or were culled from herds and flocks in Dyfed. In view of this it was considered likely that at least some of the hounds were infected with cestodes derived from these sources and it was decided to examine as many packs as possible for cestode infection. The results of the examination have been published (Williams 1976b) but a more detailed account is now presented.

MATERIALS AND METHODS

The Masters of Foxhounds (MFH) in charge of six Hunt Association packs were approached and the purpose and method of examination was explained to them. It was agreed that all six packs should be examined, but because of claims for damages two were examined some two months later than the other four. The packs were A, B, I, J, X and Y (Table 1).

A number of "farmers packs" (C - H, Table 1) had been formed in the county during the previous 10 - 15 years. These packs were formed and maintained by groups of farmers with the object of controlling foxes in areas

where Hunt Association packs did not operate. Some but not all received financial support from the Forestry Commission, farming unions and other organisations. In contrast to the Hunt Association packs the hounds were not followed by mounted huntsmen and huntswomen, but they were used for flushing foxes out of woods, copses or thickets, which were then shot by strategically placed farmers and other individuals. One of the packs, owned and managed by a single farmer and subsidised by a large grant from the Forestry Commission and owners of private tree plantations operated almost exclusively on their properties, with the farmer and sometimes his wife as the only followers, in this case, mounted.

The hounds were treated with Cestarsol at the recommended rate. As the hounds were examined during the hunting season (Fig 2), they were dosed with Cestarsol on the day following a hunt, some 16 hours after they had been fed on returning to the kennels. It was not possible to tether individual hounds nor was it possible to confine them individually. They were therefore confined in groups of up to 10 or 15 in pens with a concrete floor. After treatment they were observed and hounds that purged removed from the pen. Unfortunately it was not possible to identify the purged faeces with a particular hound.

Faeces were collected and examined by the same method described in Chapter 3.4. Details of feeding and anthelmintic treatment were collected during the visits.

RESULTS

Cestodes All packs of hounds were examined between November 1974 and March 1975.

Purging commenced between 45 and 60 minutes after treatment and all hounds which were successfully purged did so within two hours. The number of hounds in each pack and the number purged in each are shown in Table 1. The overall efficiency of purgations in all 12 packs was 95%, with a range of 87.5 - 97.05%. There was no significant difference in the efficiency of purgation between the packs ($X^2 = 7.31$ $p > 0.05$).

Of the 552 hounds purged, 381 (69%) were parasitised by one or more species of cestode. Two packs, I and J were completely free, but the proportion of infected hounds in each of the remaining 10 varied from 50% to 100%, which is illustrated in Fig 1. There was a significant difference in the infection rates between the packs even after exclusion of I and J ($X^2 = 39.5$ $p < 0.001$). The

prevalence rates of each cestode in the 552 hounds together with the number of infected packs are set out in Table 2.

If the two cestode free packs I and J are excluded then the proportions of hounds infected were rather higher (Table 3).

The distribution of each cestode species in the 10 infected packs is shown in Table 4.

A typical burden of cestodes recovered from a single hound is illustrated at Fig 3(b) in Chapter 3.4. Of the species which use the sheep as its intermediate host the commonest was T. hydatigena with between 43 and 77% of hounds in 10 packs infected. E. granulosus was identified in hounds in 8 packs with infection rates of between 27 and 52%. T. multiceps was identified in four packs with infection rates of 17 - 21% and T. ovis in 6 packs, with infection rates of 7 - 15% (Table 4).

Of the two species with intermediate stages in rodents and lagomorphs, T. pisiformis was the commonest with hounds in 3 packs infected and a nearly uniform prevalence rate of 33% - 37%. T. serialis was found in 6 hounds (1%) in 2

packs only at a prevalence rate of 2% and 10%. Twenty four hounds in two packs were infected with D. caninum (Table 4).

The infection rates between packs of hound with particular cestode infections are set out in Table 5.

There was a significant difference in the infection rates of E. granulosus ($X^2 = 26.8$ $p < 0.01$), T. hydatigena ($X^2 20.7$ $p < 0.05$) and D. caninum ($X^2 26.0$ $p < 0.01$) between the infected packs, but the differences in infection rates for the other four cestode species were not significant.

The frequency distribution of the worm burdens of each cestode and the mean in all the hounds from all the packs is set out in Table 6.

The worm burden of E. granulosus probably conforms with a negative binomial distribution but with a number of secondary peaks, one at 11 - 50 and others at 401 - 500 and 901 - 1000 parasites.

Feeding. The source and types of meat and offal fed to all the hound packs are summarised in Table 7.

Of the 6 Hunt Association packs only two, I and J were fed meat and offal from horses and cattle only. The huntsman or his kennelmen collected horse and cattle carcasses predominantly from members of the Hunt Association. The offals were only fed when supplies of carcase meat were restricted and were cooked before feeding, but the carcase meat was fed uncooked. Biscuits or biscuit meal were also fed.

The remaining Hunt Association packs were fed mainly on sheep meat and offal from carcasses either delivered by local farmers or collected by the kennelmen. From time to time cattle carcasses were collected and calf carcasses were delivered. When only a restricted supply of sheep and cattle carcasses were available, condemned offals and paunches from abattoirs and meat and offal from a knackery were obtained. None of the meat or offal was cooked before feeding. Flaked maize and biscuits or biscuit meal were also included in the diet.

All the "farmers" packs were to a large extent dependent upon the delivery of sheep carcasses and the occasional calf carcase by farmers. No regular collection service was provided by the kennelman who was usually a smallholder. When the supply of carcasses was insufficient, condemned offal from abattoirs or carcase

meat and offal from a knackery were acquired. The meat and offal were supplemented with flaked maize or biscuit meal.

Anthelmintic treatment Details of anthelmintic treatment for each pack of hounds are shown in Table 8.

Regular bunamidine treatment of all hounds was practised in only two packs I and J. There was no regular treatment programme for the other four Hunt Association packs. Treatment was restricted to the period outside the hunting season, usually when tapeworm segments were seen in hound faeces or when hounds were seen to be unthrifty. Thus the packs were treated once or possibly twice a year with bunamidine or arecoline-acetarsol. None of the other packs were treated on a regular basis, treatment with arecoline-acetarsol was confined to unthrifty hounds or those passing tapeworm segments, during the summer months when the packs were not engaged in hunting.

DISCUSSION

The overall efficiency of purgation in the 12 packs was 95% compared with 97.5% recorded by Cook (1965). There was a significant difference ($X^2 = 8.95$ $p < 0.01$) in the purgation rate compared with that recorded in dogs on the sheep rearing farms (76.9%) and a more significant

difference ($X^2 = 17.3$ $p < 0.001$) when compared with the purgation rate (76.9%) in dogs on the wintering farms. There was no clear explanation why this should be, but two factors may have been important. The hounds were segregated after treatment in groups and not tethered or confined individually as dogs were on the farms. It is known that dogs, hounds and other animals are greatly influenced by the behaviour of others in the group and the presence of one or two defaecating hounds would have stimulated the remainder to do the same. Another perhaps more important factor was the longer fasting period before treatment in hounds, which was not less than 16 hours, six to eight hours longer than for the farm dogs. There was no significant difference in the purgation rates for individual packs ($X^2 = 7.3$ $p > 0.05$).

The high prevalence rates for taeniid cestodes found in this survey (Fig 1) were similar to that reported by Cook (1965) and Walters (1977) for hounds in other parts of south and mid Wales. This was in contrast to the much lower prevalence recorded in hounds in England (Cook 1985) and north Wales (Edwards, Hackett and Herbert 1979b). The variation in prevalence rate in hounds must have reflected the extent of metacestode infections in farm livestock, particularly sheep, in Dyfed.

The species of cestodes recovered from the hounds were the same as those found by Cook (1965). Although the prevalence of cestodes in hounds in north Wales was lower, the same species apart from E. granulosus and T. serialis were identified by Edwards, Hackett and Herbert (1979).

In the present survey T. hydatigena was the most common cestode in hounds (Tables 4 & 5). Thus as indicated in the previous chapter, the frequency with which the metacestode, Cysticercus tenuicollis occurred in sheep in Dyfed was reflected in the high prevalence rates in dogs and now hounds.

E. granulosus was the second commonest cestode (Tables 4 & 5) with eight packs infected. The percentage incidence (Tables 4 & 5) was somewhat higher than 30.5% infection rate found by Cook (1965) and 28.8% recorded by Thompson and Smyth (1974, 1975) in 11/21 packs they examined. In the present survey it was known that heavier than normal losses had occurred in older sheep, especially pregnant ewes during the autumn and early winter of 1974. Many of the ewes should have been drafted from flocks in Dyfed, but because of the depressed sheep market, they were not sold, indeed in some cases could not be sold. Ewes five years of age or more have a high incidence of hydatid cysts and often reach 30% or more (unpublished data Aberystwyth and Carmarthen Veterinary Investigation

Centres). Therefore it was possible that the higher prevalence of E. granulosus in hounds may in part have been due to the consumption of offal from such ewes.

The percentage incidence in hounds parasitised by T. multiceps (Tables 4 & 5) was much higher than the 4.1% recorded by Cook (1965) and Edwards, Hackett and Herbert (1979b). Further enquiry of the kennelmen of all four packs revealed that all had from time to time received the carcasses of "giddy" sheep, namely those affected with coenurosis and that at least 20 sheep carcasses known to have died from coenurosis on one farm had been consumed by the hounds in pack C during the three or four months prior to the examination.

The prevalence of T. ovis was rather higher than that recorded in dogs on the sheep rearing farms. It was also higher than the 1.6% and 4.3% recorded by Cook (1965) and Edwards, Hackett and Herbert (1979b) respectively.

The relatively high incidence of T. pisiformis (Tables 4 & 5) is not surprising as rats which are infected with the metacestode of this parasite were attracted to the premises by the large volume of carcasses and offal handled. There was no effective rodent control programme; rats were intermittently trapped and their carcasses thrown

into the kennels or else when rats were extremely numerous one or two hounds were released into the surrounding yards overnight to specifically catch and kill them.

The presence of T. serialis (Tables 4 & 5) could be explained by the fact that occasionally during a hunt rabbits or hares were caught and consumed by the hounds. The occurrence of D. caninum, however, was much lower than might have been expected in view of the fact that its intermediate host is the dog flea, Ctenocephalides canis.

The association between the feeding of uncooked carcase meat and offal, especially of meat and offal derived from sheep and cestode infection in dog was unequivocal (3.4). No attempt was made by the attendants of hounds in the 10 infected packs, to remove obviously infected material or to trim the infected material from the affected viscera. Heads were skinned with the remainder of the carcase and thrown into the pens or yards, where one hound, usually after a confrontation with another or others took possession. Hounds in Pack G were often fed whole carcasses of sheep and calves after they had been skinned.

Special reference must be made to the circumstances which prevailed for sheep farmers in 1974 and 1975. In August 1974 the Agricultural and Advisory Service (ADAS) of MAFF issue the following statement:

Judging from reports received from many parts of the country, it is evident that fodder supplies are likely to be below normal and of below average quality. It is also expected that a likely short fall in fodder supplies has been exacerbated to some extent by retention of cattle which would normally have been sold. The situation varies considerably in different areas, but it is particularly difficult in the western and northern regions of England and in Wales. First crops of hay and silage are lower than average and quality in those regions particularly poor.

Fortunately the weather since June has encouraged second cuts and permitted later sown forage crops to be grown. It is hoped that farmers have sown more brassica crops than usual and are making effective use of fertilisers to increase late season crops and to extend the grazing season as late as practical, especially for dry and store stock." (Anon 1974).

During late 1973 and early 1974 stock prices had slumped dramatically and this continued into the summer of that year. The spring of 1974 was exceptionally dry but heavier than expected rainfall fell in June, July and August which made fodder harvesting impossible, especially in the northern part of Dyfed. Because of this and the depressed livestock sector, farmers failed to sell not only cattle, but also sheep. Consequently deaths in sheep and cattle from dietary/nutritional and metabolic problems were much higher than normal, and the volume of carcasses reaching the knackeries was so exceptionally high, that they refused to accept carcasses for a period of weeks until the accumulated meat, bones and offal could be

collected and processed by renderers. The pressure on hound kennels to accept carcasses not only of sheep but cattle increased dramatically, so that the handling of these carcasses was not as careful as it might have been under normal conditions (Fig 2a). Nevertheless the adoption of a policy of feeding cooked meat and offal together with regular anthelmintic treatment would have reduced the level of parasitism in the 10 infected packs significantly.

Stallbaumer (1965) reports that knackeries in north Wales collected sheep carcasses within a period of twenty four hours at no cost. In Dyfed in 1975-1976 six knackeries were in operation but none of these collected sheep carcasses from farms unless carcasses of cattle and/or horses were collected from the same farm or they could be conveniently picked up when journeys to other farms were made. The hound kennels therefore were a convenient and often the only place where carcasses could be disposed.

Thompson and Smyth (1975) during their examination of hounds for evidence of E. granulosus infection in their epizootiological study of equine hydatidosis in Great Britain, related the level of infection to the financial standing of pack owners; the wealthier owners were able to afford commercial dog foods and equipment for cooking offal. Of the 6 Hunt Association packs studied in Dyfed,

two kennels were equiped with boilers for cooking offal and the remainder were considering acquisition of similar equipment at the time of the examination of the hounds.

In only two of the Hunt Association packs, I & J, were the hounds regularly treated throughout the year (Table 8) and this together with the feeding of uncooked carcase meat and cooked offal (Table 7) appeared to eliminate or prevent cestode infections (Fig 1). In the remaining four Hunt Association packs, hounds were only occasionally treated and then only when they were not being hunted. The perceptions of the huntsmen and kennelmen were that such treatment was adequate and in any case treatment during the season would affect hound fitness, albeit temporarily. Those in charge of the "Farmers" packs believed that it was only necessary to treat hounds either visibly affected ie those passing tapeworm segments, or those that were unthrifty and they were oblivious to the fact that it was not always safe to assume that dogs were not infected if tapeworm segments were not shed.

Huntsmen and kennelmen were unaware that hounds disseminated cestode parasites widely over the areas they hunt over. Thompson and Smyth (1975) and Edwards, Hackett and Herbert (1979b) emphasise this. It should be pointed out however that the greatest danger of dissemination is not during the actual hunt, when dogs seldom if ever

defaecate. In the author's experience most of the dissemination occurs on fields where the hounds are confined during the time the hunt assembles. The hounds when released from the horse boxes or trailers and immediately urinate and defaecate. Often the fields used for assembly are those near a farmstead used for lambing ewes. Thus infection is disseminated on pasture just prior to the lambing period and would persist for some weeks during which lamb susceptibility is greatest.

The effect of anthelmintic treatment on immunity in hounds and indeed dogs, is unclear. It has been evident to the author and others notably, T M H Walters (personal communication) that hounds and dogs cleared of cestode infection by anthelmintic treatment rapidly became re-infected when exposed to metacestode infected material. Gemell and Soulsby (1968) report that dogs may develop an acquired immunity to cestodes which is not absolute and may wane. The occasional treatment of hounds and a rapid exposure to re-infection therefore may result in an equally rapid re-establishment of infection. It is possible therefore that the treatment of individual hounds in a pack may be more effective, allowing the majority of hounds to develop immunity, but this aspect requires further study. It transpired that all the hounds in packs C and H had been treated with arecoline-acetarsol just

prior to the commencement of the hunting season in November 1974 and these two packs were the most heavily parasitised with 94% and 100% of the hounds infected.

In summary then examination of the 12 hound packs clearly demonstrated that hunting dogs were heavily infected with cestodes derived from metacestodes in farm livestock. However care in the feeding of carcase meat and offal and regular anthelmintic treatment was capable of maintaining two cestode free packs. It was obvious that the adoption of a similar regime for the other packs would have been equally effective.

| Identity of pack | Number of hounds treated | Number of hounds purged (%) |
|---------------------|-----------------------------|--------------------------------|
| A | 68 | 66 (97) |
| B | 58 | 56 (96) |
| C | 37 | 35 (94) |
| D | 30 | 29 (97) |
| E | 32 | 30 (94) |
| F | 38 | 36 (95) |
| G | 62 | 60 (97) |
| H | 53 | 50 (94) |
| I | 40 | 35 (87.5) |
| J | 40 | 37 (92.5) |
| X | 85 | 82 (96) |
| Y | 38 | 36 (95) |
| Total | 581 | 552 (95) |

Table 1 The number of hounds treated and purged in 12 packs

| Species of cestode | No of infected hounds (%) | No of infected packs |
|----------------------|---------------------------|----------------------|
| <u>E. granulosus</u> | 162 (29) | 8 |
| <u>T. multiceps</u> | 41 (7.5) | 4 |
| <u>T. ovis</u> | 32 (6) | 6 |
| <u>T. hydatigena</u> | 270 (49) | 10 |
| <u>T. serialis</u> | 6 (1) | 2 |
| <u>T. pisiformis</u> | 53 (10) | 3 |
| <u>D. caninum</u> | 24 (4.3) | 2 |

Table 2 The prevalence of each cestode in 552 hounds from 12 packs

| Species of cestode | No of infected hounds (%) | No of infected packs |
|----------------------|---------------------------|----------------------|
| <u>E. granulosus</u> | 162 (34) | 8 |
| <u>T. multiceps</u> | 41 (8.5) | 4 |
| <u>T. ovis</u> | 32 (6.6) | 6 |
| <u>T. hydatigena</u> | 270 (56) | 10 |
| <u>T. serialis</u> | 6 (1.2) | 2 |
| <u>T. pisiformis</u> | 53 (11) | 3 |
| <u>D. caninum</u> | 24 (5.0) | 2 |

($X^2 = 39.5$ df 9 $p < 0.001$)

Table 3 The prevalence of each cestode in 480 hounds in 10 infected packs

| Species of cestode | Number (%) of hounds infected with each cestode species | | | | | | | | | | Total no. of hounds infected (%) |
|-----------------------------|---|-----------|---------|---------|---------|---------|---------|---------|---------|---------|----------------------------------|
| | Pack A | Pack B | Pack C | Pack D | Pack E | Pack F | Pack G | Pack H | Pack X | Pack Y | |
| <u>E. granulosus</u> | 24 (36) | 20 (36) | 0 | 0 | 8 (27) | 10 | 29 (48) | 23 (46) | 29 (35) | 19 (52) | 162 (34) |
| <u>T. multiceps</u> | 0 | 10 (17.8) | 6 (17) | 0 | 0 | 0 | 0 | 0 | 17 (21) | 7 (19) | 41 (8.5) |
| <u>T. ovis</u> | 6 (9) | 6 (11) | 3 (9) | 0 | 2 (7) | 0 | 9 (15) | 0 | 6 (7) | | 32 (6.6) |
| <u>T. hydatigena</u> | 32 (49) | 28 (50) | 20 (57) | 16 (55) | 13 (43) | 17 (47) | 39 (65) | 36 (72) | 41 (50) | 28 (77) | 270 (56) |
| <u>T. serialis</u> | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 5 (16) | 0 | 0 | 6 (1.2) |
| <u>T. pisiformis</u> | 22 (33) | 0 | 13 (37) | 0 | 0 | 0 | 0 | 18 (36) | 0 | 0 | 53 (11.0) |
| <u>D. caninum</u> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 (6) | 0 | 21 (58) | 24 (5) |
| Number of hounds purged (%) | 66 | 56 | 35 | 29 | 30 | 36 | 60 | 50 | 82 | 36 | 480 |

Table 4 The distribution of cestode species in 480 hounds in 10 infected packs

| Species of cestode | No of packs | Total no of infected/purged hounds in in- fected packs (%) | X bet- ween in- fected packs | Signi- ficance |
|-----------------------|----------------|---|---------------------------------------|-------------------|
| <u>E. granulosus</u> | 8 | 162/416 (39) | 26.8 | p < 0.001 |
| <u>T. multiceps</u> | 4 | 41/209 (19.6) | 0.18 | NS |
| <u>T. ovis</u> | 6 | 32/276 (11.6) | 4.49 | NS |
| <u>T. hydatigena</u> | 10 | 270/480 (56.2) | 20.7 | p < 0.05 |
| <u>T. serialis</u> | 2 | 6/110 (5.5) | 2.23 | NS |
| <u>T. pisiformis</u> | 3 | 53/151 (35.1) | 0.17 | NS |
| <u>D. caninum</u> | 2 | 24/86 (27.9) | 26.0 | p < 0.01 |

Table 5 Infection rates of cestode species in all packs

| Species of cestode | No of hounds not inftd | No of hounds with worm burdens of | | | | | | | | | | Mean* |
|---------------------|------------------------|-----------------------------------|----|----|----|----|------|-------|-------|-------|--|-------|
| | | 1 | 2 | 3 | 4 | 5 | 6-10 | 11-20 | 21-30 | 31-50 | | |
| <u>T multiceps</u> | 511 | | | | | | 31 | 9 | 1 | | | 9.4 |
| <u>T ovis</u> | 520 | 11 | 11 | 8 | | | 2 | | | | | 2.2 |
| <u>T hydatigena</u> | 282 | 46 | 58 | 63 | 61 | 23 | 12 | 5 | 1 | 1 | | 3.9 |
| <u>T serialis</u> | 546 | | 5 | | 1 | | | | | | | 2.3 |
| <u>T pisiformis</u> | 499 | 4 | 13 | 20 | 14 | | 2 | | | | | 3.0 |
| <u>D caninum</u> | 528 | 3 | 10 | 9 | 2 | | | | | | | 2.4 |

E granulosus

| No of hounds not infected | No of hounds with worm burdens of | | | | | Mean* |
|---------------------------|-----------------------------------|----------|-----------|---------|---------|-------|
| | 1-10 | 11-50 | 51-100 | 101-200 | 201-300 | |
| 391 | 11 | 30 | 3 | 7 | 14 | |
| | 301-400 | 401-500 | 501-600 | 601-700 | 701-800 | |
| | 22 | 26 | 13 | 7 | 6 | |
| | 801-900 | 901-1000 | 1001-1500 | 5700 | | |
| | 6 | 13 | 2 | 1 | | 382 |

* Mean per infected dog

Table 6 Frequency distribution of cestode species worm burdens in 552 hounds grouped from all packs

| Identity of packs | Source of meat and offal | Cooked/uncooked |
|-----------------------------------|--|-----------------|
| Hunt Association packs I and J | Horse and cattle carcase meat | Uncooked |
| | Horse and cattle offal | Cooked |
| Hunt Association packs A B X Y | Cattle and sheep carcase meat and offal | Uncooked |
| | Condemned meat and offal from abattoirs | Uncooked |
| | Knackery meat and offal | Uncooked |
| Farmers packs C D E F G H | Sheep carcasses and offal | Uncooked |
| | Knackery meat and offals | Uncooked |
| | Abattoir waste | Uncooked |

Table 7 Sources of meat and offal fed to 12 packs of hounds

| Identity of Pack | Frequency of treatment | Anthelmintic |
|-----------------------------------|--|--|
| Hunt Association packs I and J | Three monthly intervals | Bunamidine |
| Hunt Association packs A B X Y | Occasional | Bunamidine or Arecoline- acetarsol |
| "Farmers" packs C D E F G H | Occasional treatment of individual hounds | Arecoline- acetarsol |

Table 8 Frequency of anthelmintic treatment of 12 hound packs

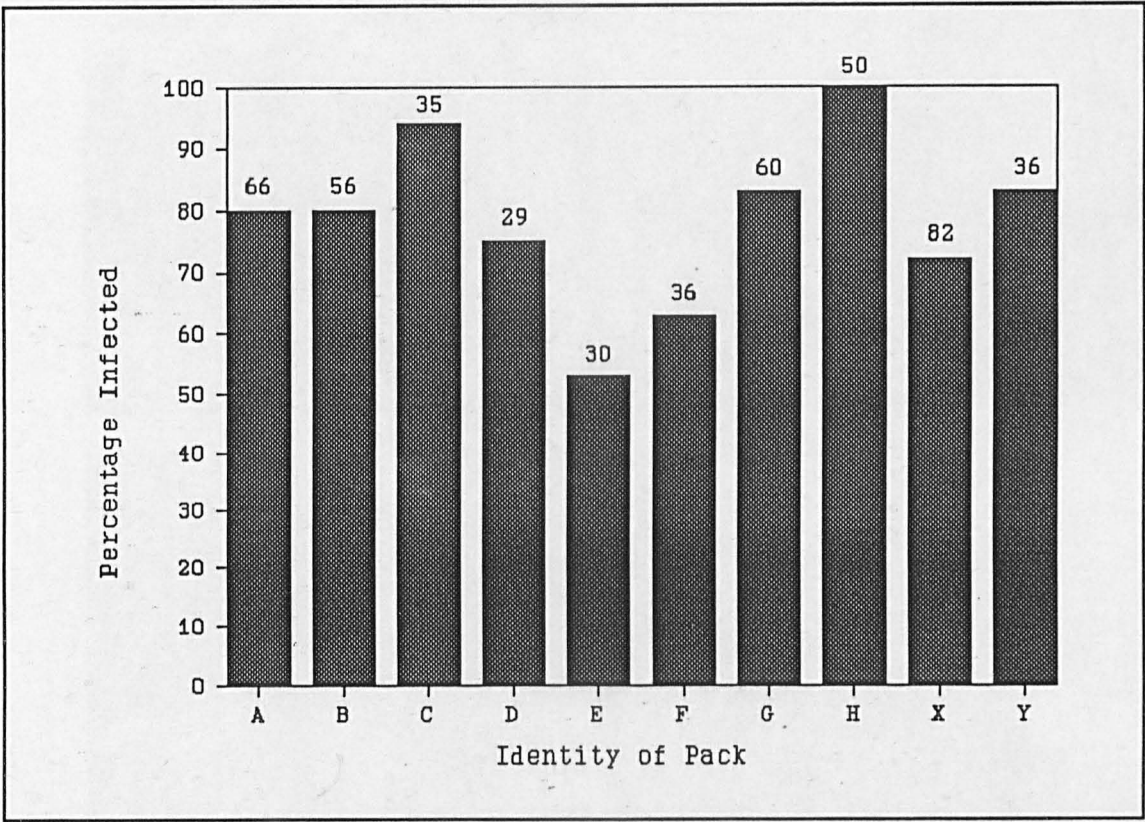


Figure 1 : Percentage of cestode infected hounds in the 10 infected packs and number of hounds purged in each pack.

**Fig 2 Accommodation and anthelmintic
treatment of fox hounds**

a) The yard and buildings on an
abandoned farmstead used by Pack B.
Note the sheep carcasses left by
farmers overnight

b) Individual hounds in Pack B
being segregated prior to treatment



3.6 The cestodes of the red fox (Vulpes vulpes) and their relationship with diet

There is relatively little published information on the role of the red fox in the transmission of tissue cestodes to ruminants in Great Britain. Cook (1965) concluded after the examination of foxes from a number of areas in Great Britain, that although the fox harboured a number of cestode species, the only species that developed to maturity was E. granulosus and therefore this particular species was the only one transmitted to farm livestock and other animals.

In view of the paucity of information available it was decided to examine as many foxes as possible for the presence of cestodes, especially T. multiceps and thereby assess the importance of the fox in the epizootiology of metacestode infection in farm livestock. The results of a preliminary study had been published (Williams 1976c) in which all the helminths recovered from 149 foxes were identified. This study was extended and at the same time an attempt was made to analyse the stomach contents of foxes and identify as far as possible the extent to which foxes fed on sheep carcasses and other ovine material. Thus it would be possible to relate the cestode species with food items consumed.

MATERIALS AND METHODS

Fox carcasses were collected from a number of sources. The vast majority were shot by followers of farmers' hound packs. Others were submitted through the good offices of Mr G Lloyd, a fox ecologist employed by MAFF, by individual farmers and groups of farmers operating a fox control programme on their holdings. Other farmers who learnt of the survey informed the laboratory when foxes had been snared or shot. Approximately 30% of the total submitted had been snared and the remainder shot. Nearly half the total originated from the Cilycwm, Farmers, Llandovery, Llanwrda and Rhandirmwyn districts of Dyfed and the remainder from other areas of the county.

The carcasses were collected and subjected to a post mortem examination within 24 hours of death. No attempt was made to estimate accurately the age of individual foxes, but they were classified as immature or mature on the basis of body weight and permanent teeth eruption. Immature foxes classed as such were considered to be under 9 months of age. The sex of each specimen was also recorded.

The gastro-intestinal tracts were removed from the carcasses and the small and large intestines separated from the stomach. A bowel scissors was then used to open the small intestine along its whole length and the contents were gently washed into a plastic bowl. A blunt knife or

a spatula was used to scrape the mucosa and the scrapings added to the intestinal contents, which were then washed through metal sieves of 60 and 100 meshes to the inch in a similar manner to that described for the examination of dog and hound faeces. All the helminths were stored in 5 - 10% formalin until they could be identified at a later date.

The stomach contents were transferred into kilner jars and either stored in deep freeze at -15°C or preserved by the addition of an equal volume of 10% formal saline, to await analysis. Food items were identified in a similar manner to that described by Coman and Brunner (1972). Extensive use was made of known specimens of small mammals, especially their skeletons for comparison. Often the only practical means of identifying mammalian remains was by the examination of individual bones, feet and teeth, or through the examination of hair structure. Whole mounts or cross sections of hair or fibres were prepared and examined microscopically, as described by Appleyard (1960).

RESULTS

A total of 387 foxes were submitted and examined between March 1972 and March 1975, but the vast majority were received during the autumn - spring period (October to April). Fifty eight (15%) were classified as immature and 201 (51.9%) female and 186 (48.1%) male.

Cestodes Tapeworms were found in the intestines of 58 (14.9%) foxes and a total of eight species were identified. Fig 3 Chapter 3.4 illustrates the cestodes recovered from one fox and individual cestode species from foxes are illustrated in Fig 1 of this chapter. The infections were usually of more than one species, but in some instances when only one species was identified, fragments of other species may have been discarded because the scolices had become detached from the strobila and were excluded from the data. The distribution of the various species is set out in Table 1.

The commonest species were T. hydatigena and T. pisiformis in both immature and mature foxes. E. granulosus was identified in both immature and mature foxes but T. ovis and T. multiceps in mature specimens only. The remaining three species were T. polyacantha, T. serialis and T. taeniformis.

The frequency distribution of worm burdens of each cestode in the foxes are set out in Table 2. There were no significant differences in the burdens between males and females or immature and mature foxes and therefore the data have been pooled.

Food items The stomachs of 107/387 foxes were completely empty and no attempt was made to identify food items in the intestine of these animals. Varying amounts of food were found in the stomachs of the remainder and the main seven categories are listed in Table 3.

Food material of mammalian origin was found in 221/280 (78%) of the stomach contents and the various mammalian species are set out in Table 4.

Of the mammalian species identified rabbit and hare remains were the commonest (Table 4). Besides hair, muscle, long bones and vertebrae, other remains included skull fragments, portions of ear pinnae, teeth, feet and occasionally faecal pellets. Other items identified were from rats, mice and voles (Table 4).

Food items of ovine origin were also identified in the stomach contents of foxes (Table 4). The most frequently identified sheep material was wool, but muscle, portions of viscera, bones, hooves of lambs and placenta were also

identified with placentae and lamb hooves being especially common during the period January to March. Most of the sheep material in late winter early spring appeared to have been derived from stillborn or aborted lambs, recognisable from the appearance of the unexpanded lungs, "slipper" hooves and skin with no or very little wool cover. The muscle and viscera from older sheep had undergone decomposition prior to ingestion.

Food items of avian origin including eggs were identified, whilst cold blooded vertebrates, insects and other vertebrates were also found in many foxes (Table 4). Vegetable matter and other miscellaneous items included sandwiches, sometimes ingested with their cellophane wrappers, cooked meat and cheese which presumably had been discarded by tourists or holiday makers. Apples, pears and plums were often found in foxes examined during the summer months and an aged male with severe gingivitis had existed on an exclusive diet of blackberries for a period of several days if not longer.

DISCUSSION

The percentage of foxes parasitised by all cestode (Table 1) was much lower than the 25% recorded by Cook (1965) for foxes trapped in mid and south Wales. Edwards, Hackett and Herbert (1979b) reported that 19% of the foxes they

examined from north Wales were infected by taeniid cestodes, but they only identified two species in the 111 specimens. Cook (1965) also only identified two species in a small number of foxes from Carmarthen.

Mature and immature cestodes of all eight species were identified in the foxes, but the numbers and the strobilar lengths were far smaller than for the same species recovered from dogs and hounds, indicating that the environment of the fox intestine was not as favourable for the establishment and development of the parasite as the intestine of the dog and hound. Cook (1965) claimed that the only cestode that matures in the small intestine of the fox and therefore is the only one that is capable of being transmitted by foxes is E. granulosus. Such a claim was invalidated by the present study and subsequent studies by Hackett and Walters (1980b) and Edwards, Hackett and Herbert (1979b) confirm that cestodes other than E. granulosus mature in the fox and are therefore capable of being transmitted by it.

Of particular interest was the prevalence of cestode species with intermediate stages in sheep. By far the commonest of these was T. hydatigena. As reported in earlier chapters, the metacestode of this parasite is common in sheep in Dyfed and foxes scavenging on sheep carcasses were often exposed to infection. Twenty three

foxes infected with this cestode were submitted from areas within about 2.5 kilometres of a knackery and this is further discussed later in this chapter.

The low prevalence of T. ovis in the foxes reflected the low prevalence of the metacestode, Cysticercus ovis in sheep in Dyfed. The failure of Hackett and Walters (1980b) and Edwards, Hackett and Herbert (1979b) to demonstrate the parasite in foxes indicates that it is either absent, or at a very low prevalence in sheep in north and mid Wales.

It was assumed that the subspecies of E. granulosus recovered from the foxes was E. granulosus granulosus, because they had opportunities to scavenge almost exclusively on the sheep. However Cook (1989) claimed on the basis of a prolonged programmed of experimental work with metacestodes derived from sheep and horses in Great Britain, that the only subspecies existing was E. granulosus equinus. McManus, Thompson and Lymbery (1989) and Lymbery and Thompson (1989) refuted this claim, stating that his interpretation of the experimental work was erroneous, especially as Thompson (1985) had demonstrated that E. granulosus granulosus was capable of infecting foxes as well as dogs. It was therefore likely that the specimens recovered were of the subspecies E. granulosus granulosus.

The identification of T. multiceps was unexpected because Cook (1965) and Mr G Lloyd MAFF fox ecologist believed the jaw strengths of foxes was much weaker than that of dogs and therefore foxes would be unable to gain access to the brain of sheep. Nevertheless such access is not difficult in sheep affected with chronic coenurosis because the frontal bones may undergo considerable pressure atrophy and be paper thin.

The presence of cestodes namely T. pisiformis, T. polyacantha, T. serialis and T. taeniformis with intermediate stages in lagomorphs and rodents was to be expected. Although T. polyacantha had been previously identified in foxes from the European continent, this was the first identification from foxes in Great Britain (Mr S Prudhoe, personal communication).

Examination of the stomach contents of the 280 foxes confirmed the findings of McIntosh (1963), Fairley (1965, 1966) and Coman (1973), that the fox has a varied diet which is dictated by the availability of food items. In this study and the others quoted, rabbits and/or hares, mice and rats contributed greatly to the diet. However the presence of ovine material in 21% of those stomachs which included mammalian remains (Table 4) indicated that ovine material was ingested when readily available. In this context reference should be made to comments made by

by several farmers who lambed their flocks indoors. It was customary for placentae to be removed from lambing pens and stored outside lambing sheds until they could be buried. Unless they were placed under cover foxes would remove them and one farmer recalled that in his experience foxes remained in the vicinity of lambing sheds at night during the lambing period. Similarly aborted or stillborn lambs were removed from outside lambing sheds and undoubtedly placentae and aborted or stillborn lambs were scavenged on pastures.

It was noteworthy that 23 of the 46 foxes, with ingested food items of ovine origin, had been shot or snared within 2.5 kilometres of a knackery, where farmers regularly deposited sheep carcasses at night when operations for the day had ceased. The carcasses were left on a concrete apron outside the processing building and the knackery owner reported that invariably the carcasses had been scavenged at night and the abdomen penetrated. The prevalence of T. hydatigena in the 23 foxes could therefore be explained by the availability of carcasses at the knackery.

McIntosh (1963) stated that in his view the fox was not only an opportunist scavenger but also an opportunist predator. Alexander, Mann, Mulhearn, Rowley, Williams and Winn (1967) in studying the activities and behaviour of foxes concluded that few kill sheep and are intimidated

even by lambs of 3 - 4 months of age. There have been few confirmed reports of foxes attacking adult sheep or lambs in Great Britain and the televised programme based on the Nature Conservancy Council Study shown on BBC 1 in 1990 indicated that in the north of Scotland, foxes did not attack sheep or lambs. The author knows of only one confirmed incident of fox predation on sheep which occurred in the spring of 1963 in Powys. Two foxes isolated a ewe and her twin lambs from the rest of the flock and were successful in killing and removing one of the lambs. This incident was filmed at the time.

The variety of food items in the stomachs of the foxes confirmed that they were opportunist feeders and adapted their feeding habits to the availability of food items. It is also evident that the cestode infections identified mirrored the food items consumed and that although cestodes with intermediate stages in sheep matured in foxes they only played a minor role in their transmission to sheep compared with farm dogs and hunting dogs.

| Species of cestode | Number of immature foxes infected (%) | Number of mature foxes infected (%) | Total number(%) |
|-----------------------|--|--|--------------------|
| <hr/> | | | |
| <u>E. granulosus</u> | 2 (3.4) | 5 (1.5) | 7 |
| <u>T. multiceps</u> | - | 1 (0.3) | 1 (0.2) |
| <u>T. ovis</u> | - | 3 (0.9) | 3 (0.8) |
| <u>T. hydatigena</u> | 3 (5.1) | 30 (9.1) | 33 (8.5) |
| <u>T. serialis</u> | 1 (1.7) | 3 (0.9) | 4 (1.0) |
| <u>T. pisiformis</u> | 12 (21) | 26 (7.9) | 38 (10) |
| <u>T. taeniformis</u> | 2 (3.4) | 3 (0.9) | 5 (1.3) |
| <u>T. polyacantha</u> | 2 (3.4) | 3 (0.9) | 5 (1.3) |
| All species | | | 58 (14.9) |

Table 1 The distribution of cestode species recovered from 58
immature and mature foxes

| Cestode species | Number of foxes with worm burdens of | | | | | | | | | | Mean burden* |
|--------------------|--------------------------------------|---|---|---|---|---|------|-------|-------|-------|-----------------|
| | Neg | 1 | 2 | 3 | 4 | 5 | 6-10 | 11-20 | 21-30 | 31-50 | |

| | | | | | | | | | | | |
|------------------|-----|--|----|----|---|----|---|----|---|---|------|
| <u>E. gran.</u> | 380 | | | | | | | 2 | 4 | 1 | 22.4 |
| <u>T. mult.</u> | 386 | | | 1 | | | | | | | 3 |
| <u>T. hydat.</u> | 354 | | 12 | 12 | 7 | | 2 | | | | 3.2 |
| <u>T. ovis</u> | 384 | | 1 | | 2 | | | | | | 3.3 |
| <u>T. pisif.</u> | 349 | | 4 | 2 | 1 | 11 | 4 | 11 | 5 | | 8.9 |
| <u>T. taeni.</u> | 382 | | | | | 3 | 1 | 1 | | | 7.0 |
| <u>T. poly.</u> | 383 | | | | | 3 | 2 | | | | 5.6 |
| <u>T. seri.</u> | 383 | | | | | | 4 | | | | 7.0 |

* Per infected fox

Table 2 Frequency distribution of the worm burdens of cestode species in 387 foxes

| Food category | Number of stomachs contents positive (%) |
|--------------------------|---|
| Mammals | 221 (78) |
| Birds | 66 (23.7) |
| Cold blooded vertebrates | 8 (2.8) |
| Insects | 106 (38) |
| Other invertebrates | 21 (7.5) |
| Vegetable matter | 140 (5.6) |
| Other items | 34 (12) |

Table 3 Food items identified in the stomach contents of
280 foxes

| Species from which food item derived | Number of stomach contents positive (%) |
|---|--|
| Rabbits, hares | 77/221 (35) |
| Rodents | 64/221 (29) |
| Sheep | 46/221 (21) |

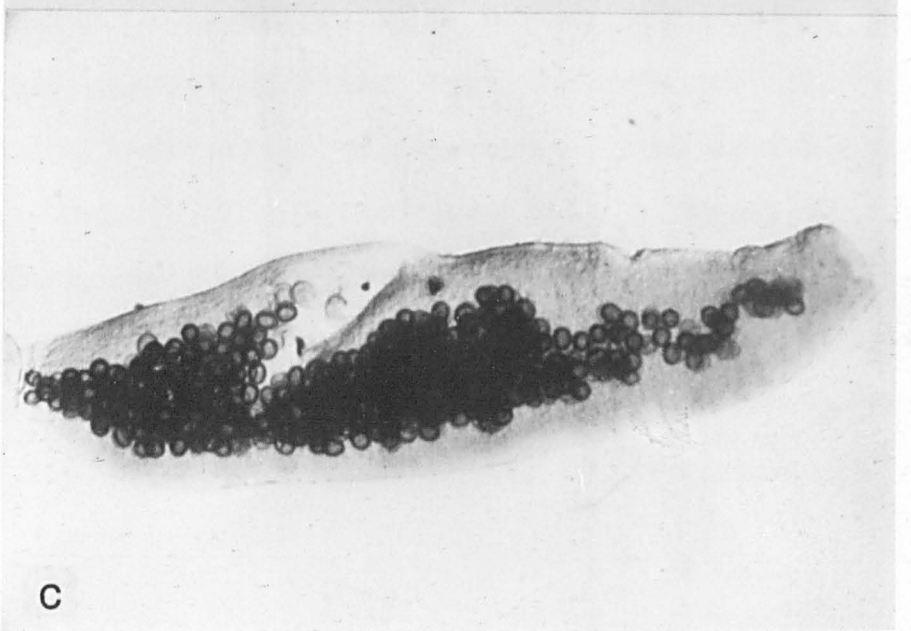
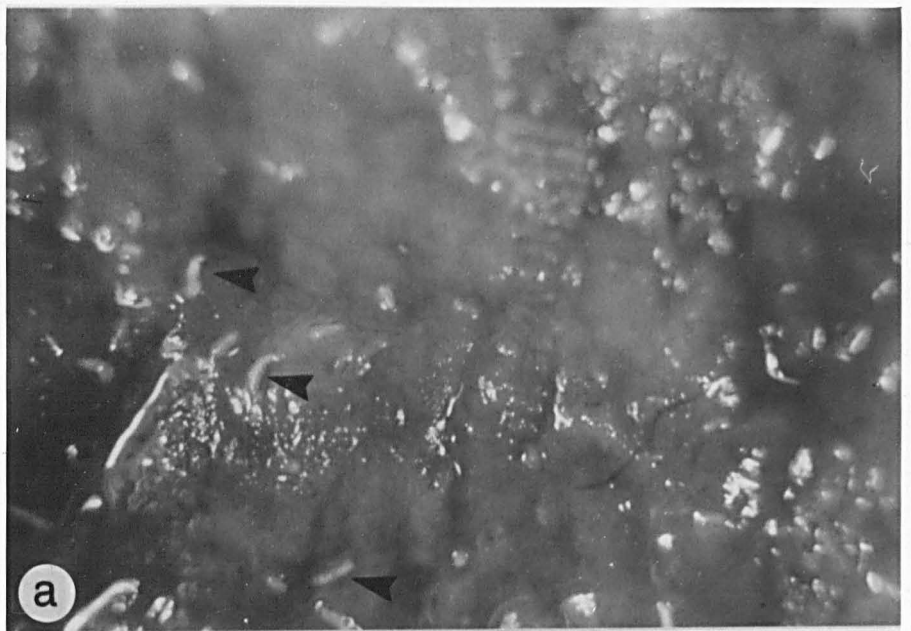
Table 4 Mammalian species from which food items were
derived in stomach contents of 221 foxes

Fig 1 Echinococcus granulosus recovered
from a fox

a) The opened small intestine of
a fox with the mature cestodes
(arrowheads) attached to the mucosa

b) The scolex and anterior
segments (x 20)

c) The gravid segment of a mature
cestode



3.7 The cestodes of pet dogs

One of the reasons suggested by farmers and representative of the farming unions for the increased occurrence of coenurosis in both sheep and cattle, was the increased ownership of dogs by the general public. It was alleged that pet dogs were exercised on common grazings or that they were allowed to stray on to enclosed grazings and this could also be linked to the sharp rise in the number of reported incidents of sheep worrying. A number of farmers whose properties had footpaths reported that a significant number of walkers were accompanied by dogs, many of whom were allowed to roam freely. In view of their concern and in the interest of embracing all aspects of the epizootiology of coenurosis, it seemed desirable to investigate the possible role of pet dogs.

MATERIALS AND METHODS

It was decided to concentrate as far as possible on pet dogs in the upper Cothi and Tywi valleys and thereby supplement the information obtained from farm dogs in the same area. Owners of pet dogs had been informed of the survey of farm dogs through conversations with farmers and several expressed an interest in participating. Farmers were asked for the names and addresses of interested owners and they were invited by letter to participate.

Veterinary surgeons were also asked for the names and addresses of pet owner clients in the area who might be interested and they too were invited to participate by letter. Practising veterinary surgeons also offered to make available for post mortem examinations the carcasses of dogs which they had destroyed or those that had died, after the owners had agreed to the post mortem examinations.

Dogs were treated with Cestarsol as described in Chapters 3.4 and 3.5. Information on the feeding, management and anthelmintic treatment was collected at the time of the visits to treat the dogs and collect faeces samples.

The carcasses of dogs were subjected to the same post mortem procedures as those used for fox carcasses (Chapter 3.6).

RESULTS

Although the owners of 184 dogs of various breeds and crosses were keenly interested and expressed a wish to participate, a number however owned holiday cottages and visited the area only occasionally, usually at weekends during the spring and summer and because of practical difficulties they were excluded from the survey.

Consequently only 157 dogs were examined for cestodes in the period June to September 1974. One hundred and thirty three dogs were from the Cilycwm and Rhandirmwyn area and 24 were owned by persons residing in the north west area of Llandovery, who were related to or connected with farmers in Cilycwm and Rhandirmwyn. Of the 157 treated, 133 (85%) were purged.

Thirty three carcasses were subjected to a post mortem examination during the same period. Twenty of these originated from Cilycwm and Rhandirmwyn, the remainder from Llandovery, Llangadog and Llandeilo.

Cestodes Tapeworms were demonstrated in the faeces of five (3.7%) dogs. Three yielded D. caninum only. Five mature specimens of T. multiceps were recovered from one dog, and one of T. hydatigena from another, both owned by a forestry worker who assisted a Cilycwm sheep farmer on a casual basis at week ends.

No cestodes were recovered from any of the 33 carcasses examined.

Feeding All 129 dog owners claimed that canned or dehydrated meat was regularly fed to their pets, but from time to time this was supplemented by meat scraps and/or bones obtained from butchers, which were fed uncooked.

The forestry worker admitted that his dogs often accompanied him when he visited the Cilycwm farm and occasionally consumed meat and offal from sheep slaughtered on the farms. He further confirmed that the head of a sheep which had shown clinical signs of coenurosis had been fed to his dogs some three months before they were treated with Cestarsol.

Management All the owners in Cilycwm and Rhandirmwyn were adamant that their dogs were confined within the perimeters of their dwellings although they were allowed to roam over the gardens and lawns. Few of the premises had what could be considered stock proof fencing or hedging, but owners were very conscious of the possibility of sheep worrying and emphasised that dogs were always exercised on leads along roads and paths.

The situation in the more urban area of north west Llandovery was rather different. Dogs, especially those owned by residents on a small council estate, were allowed to roam freely from morning to night and groups of three or four dogs were known to scavenge around cafes, bakers and butchers shops and a hotel near the centre of the town. A farmer who grazed sheep on land adjacent to the council estate had shot a dog which was seen attacking a

sheep and subsequently the police had requested all dog owners to confine their pets. Despite this request a number of dogs continued to be let loose each morning.

Anthelmintic treatment Seventy four of the 129 dog owners had sought veterinary advice on treatment for parasites during the previous 12 months and claimed that treatment had been given. Some of the remainder believed that one treatment either before or at purchase was sufficient to provide life long freedom. The majority however considered that the feeding and management of their dogs were such that only a remote chance of becoming infected with either round worms or tapeworms existed and therefore no treatment was necessary. Few owners were aware of the life cycle of cestodes.

DISCUSSION

The interest shown by pet dog owners, particularly in the rural areas was gratifying and reflected their interest in finding out whether their dogs were infected with cestodes and in maintaining a good and close relationship with the farming community. Even those who owned holiday cottages, many of whom were permanently resident in the industrial

midlands or home counties were equally interested and concerned to remain on good terms with farmers in the area.

The fact that only 3.7% of dogs were infected with cestodes was not unexpected in view of the changes in the feeding of dogs during the last three decades. It was not uncommon for the author to find that 30 - 40% of dogs in the rural areas of the old county of Carmarthen were infected with cestodes in 1950 - 1953, when dogs were fed on meat or offal condemned as unfit for human consumption or on similar items bought direct from knacker yards or pet shops supplied by knacker's yards. The fact that only two dogs were infected with cestodes of sheep or cattle origin, namely T. multiceps and T. hydatigena, was significant in that they had been deliberately fed with the meat and offal, including the head of a sheep affected with coenurosis. Thus infection of pet dogs and farm dogs with T. multiceps was associated with the deliberate feeding of heads from sheep affected with coenurosis.

As indicated above the feeding of pet dogs has undergone a remarkable transformation since the early 1960's. All the dogs owners fed either canned or dehydrated meat to their pets, which from time to time was supplemented by meat scraps and bones obtained from the butcher. None obtained meat or offal from knacker's yards or even hound kennels.

Indeed the pet shops that sold such meat in the 1960's no longer existed and most of the canned or dehydrated meat was obtained from grocery shops or pharmacists.

Pet owners in rural areas, especially in sheep farming areas made every attempt to confine dogs either by tethering or by permitting them only to roam within the perimeter fence or hedge of new dwellings. The main reason for doing so was to avoid any possibility of dogs becoming involved in sheep worrying incidents, thereby antagonising the farming community. Following the survey, these owners were also not only aware of the animal and human health implications of not feeding uncooked meat and offal to dogs, but also of the need to control dogs for the same reasons.

The fact that many owners had sought veterinary advice on treatment for parasites within the previous 12 months was rather surprising. It transpired that this had arisen because they had either taken the dogs for canine distemper vaccination or to be treated for some ailment and that treatment for parasites had arisen during a general conversation on health matters. The remaining owners considered that booster vaccinations were unnecessary as their dogs never or seldom came into direct contact with other dogs. They were not prompted therefore to treat their dogs with taeniocides. As might be

expected awareness of the animal and human health implications of cestode infections in dogs was low in this group of owners.

The survey demonstrated that although pet dogs could become infected with certain species of cestodes, the infection rate was much lower than that demonstrated in farm dogs and hounds in the same area. Therefore they did not pose a significant risk to farm livestock. It was also clearly demonstrated that the feeding of sheep heads was an important factor in the epizootiology of coenurosis.

4 EXPERIMENTAL INFECTION OF DOGS, RABBITS, MICE AND LAMBS WITH TAENIA MULTICEPS

4.1 Experimental infection of dogs

The biotic potential of Taenia species in dogs is dependent upon the prevalence of infection, the number of worms per infected dog, the rate of proglottid release and the number of eggs per proglottid (Gregory 1976). Whilst experimental infections of Taenia hydatigena and Taenia ovis had been widely studied there have been few observations on experimental infections of Taenia multiceps in the same species. In view of this and to complement the information obtained in Chapters 3.4, 3.5 and 3.7 it was considered necessary to study experimental infections in dogs to determine the time of first proglottid release, the rate and pattern of release and the longevity or duration of infection in dogs.

MATERIALS AND METHODS

Three crossbred Border Collie male puppies (Dogs No 1, 2 and 3) of 7.2, 8.4 and 7.7 kg body weight, aged between four and six months, a crossbred Alsation male puppy of 12.2 kg body weight, aged six months (No 4) and a six months old Shetland Collie bitch weighing 4.3 kg (No 5) were used for the experimental work. The puppies derived

from "worm free" bitches were isolated after weaning at approximately eight weeks, in pens with a concrete floor in a loose box at the laboratory, maintained on a diet of canned or dehydrated meat and biscuit meal, and on the basis of regular faecal monitoring were shown to be free of all helminths.

The puppies were infected as and when protoscolices became available, which were derived from fresh Coenurus cerebralis cysts removed from killed sheep affected with coenurosis, or from cysts removed surgically from clinically affected sheep. The cysts were carefully examined to confirm that the recognisable features of Coenurus cerebralis were identified and an appropriate number of protoscolices removed. The protoscolices were embedded in a small ball of lard or canned meat which was then readily ingested by the dogs. Dogs No 1 and 2 were infected simultaneously with protoscolices from the same coenurus, but the others received protoscolices derived from different coenuri.

The dogs were confined to the pens after infection, but were exercised daily for a period of twenty to thirty minutes on a lead. Each dog was regularly examined and the pens inspected and cleaned out daily. Faeces samples were collected at least once daily from day 35 post

infection until the termination of the experiment at 180 days post infection, when all the dogs were treated with Cestarsol.

Faeces samples after collection were placed in shallow black trays and initially examined for the presence of proglottids on the surface. Subsequently the faeces were broken up gently with scalpel and forceps in approximately ten times the volume of normal saline to ensure even dispersal. Soft or semi solid faeces were easily broken up and dispersed in a similar volume of saline by gentle agitation. Proglottids were readily identified against the black background by the naked eye, but when necessary any suspicious fragments were closely examined under a dissecting microscope.

RESULTS

All five dogs remained healthy during the period of observation, although No 5 developed a transient but slight diarrhoea 14 days after infection which peristed for about 72 hours. There was no decline in appetite and they continued to thrive during the experimental period.

The data obtained from the experimental infections are summarised in Table 1.

The prepatent period in dog No 1 which had been infected with two protoscolices was 42 days and proglottids were voided from that time until 140 days after infection. None were detected from then until treatment at 180 days after infection. The daily rate of proglottid release varied from 0 - 7 with an average of 1.9. No cestodes were recovered after treatment with Cestarsol.

Proglottids were first detected in the faeces of dog No 2, 51 days after infection with 5 protoscolices. Although the source of protoscolices was the same as for dog No 1 and the dogs were litter mates, the prepatent period was 9 days longer. An average of 2.1 proglottids, range 0 - 8, were voided each day until 180 days post infection when two specimens of T. multiceps were recovered after treatment with Cestarsol.

Dog No 3 was infected with five protoscolices and proglottids were voided between 61 and 180 days post infection. The average daily proglottid release was 1.7 (range 0 - 4). Two adult T. multiceps were recovered after treatment with Cestarsol.

Ten proglottids were administered to dog No 4 and proglottids were first detected in the faeces 47 days after infection and continued to be voided until 121 days

post infection. An average of 2.8 proglottids (range 0 - 16) were detected daily. No cestodes were recovered after treatment with Cestarsol.

Dog No 5 received five protoscolices and 45 days later proglottids were first detected in the faeces and continued to be voided until the infection was terminated by treatment with an average dose of Cestarsol. An average of 3.4 proglottids (range 0 - 11) were voided each day and three specimens of T. multiceps were recovered following treatment. This bitch, which had been maintained worm free through regular anthelmintic treatment and fed on canned meat and biscuit meal, was reinfected with 30 protoscolices, 8 months later. The prepatent period on this occasion was 56 days and average of 4.3 proglottids (range 1 - 21) were voided each day until 147 days post infection when a mass of live tapeworms were expelled. Nineteen gravid specimens of T. multiceps were identified in the mass, but none were recovered after treatment with Cestarsol 33 days later.

DISCUSSION

In these experiments infection was established and became patent in all the dogs. This was in contrast to the observations of Willis and Herbert (1984), who reported that the number of protoscolices which developed into

mature tapeworms in their experimental infections was variable, with between 0 - 77% establishment of infection. These authors however do not provide information on the age of their experimental dogs or indeed whether they had been previously exposed to infection, two very important factors in determining whether infection is established and matures.

The prepatent period in the five dogs varied from 42 to 61 days with an average of 49.8 days, which is in broad agreement with that recorded for other Taenia species. It was noteworthy that there was a difference of 9 days in the prepatent period of infection in two litter mates infected with protoscolices from the same coenurus. Significantly, Abassov (1965) reported that he found on post mortem examinations of dogs 56 days after infection with 50 protoscolices of T. multiceps, 10 protoscolices of T. hydatigena and 5000 Echinococcus granulosus protoscolices that 80% of T. multiceps, 28 - 60% of T. hydatigena and only 18 - 42% of E. granulosus had become gravid. He concluded that the presence of other cestodes had little effect on the number of T. multiceps that became established in the small intestine. Unfortunately he did not study the rate at which gravid proglottids were released by T. multiceps.

There appear to be no previous reports on the release of T. multiceps proglottids in dog faeces, although Willis and Herbert (1984) made such observations but did not report them. In the present study there was a marked variation in the number of proglottids voided each day and from day to day. The release of several proglottids on one day is likely to be an important factor in transmission. There appeared to be no direct relationship between the number of proglottids voided and the number of protoscolices in the infecting dose.

Willis and Herbert (1984) were first able to demonstrate T. multiceps oncospheres dispersed in the faeces between 38 and 43 days after infection, earlier than proglottids were first detected in the present study. They calculated that each gravid segment contained 37,000 oncospheres and that most of them are released into the intestinal contents when the proglottid is shed. These authors do not provide any information on whether the eggs and proglottids appear in the faeces simultaneously, but it is likely that they do so, or within a day or two. Willis and Herbert (1984) also conclude that the dispersal of oncospheres in the intestinal contents and subsequently in the faeces is as important or indeed possibly more important than the voided proglottids in the transmission of infection to sheep and cattle. They calculated that voided proglottids only contained 7.5 - 18% of the total

number of oncospheres produced by each proglottid. The availability of the oncospheres to intermediate hosts is moreover dependent upon the breakdown of proglottids and faeces. Proglottids putrefy in a matter of hours during the summer and in one to two days during the winter, whereas the faeces take much longer to disintegrate, usually two to four weeks in summer and winter (unpublished observations at the Carmarthen Veterinary Investigation Centre). Another factor which influences availability are grass growth and rainfall which will be discussed in Chapter 5.1.

In the three dogs Nos 2, 3 and in 5 (Table 1) during the initial infection, proglottids were still being released six months after infection. Penfold, Penfold and Phillips (1973) demonstrated that, although Taenia infections in dogs were self limiting, infection may extend over several years. Gregory (1976) demonstrated too that T. ovis infection in a dog persisted over a period of seven years and one month. It seems therefore that in the present study, had the three dogs not been treated, they could have remained infected for a much longer period. It is also likely that field infections of T. multiceps in some dogs may persist for months or years, rather than weeks.

The natural termination of infection in dogs Nos 1, 4 and 5 after reinfection on days 140, 121 and 147 respectively (Table 1) illustrated the immune mechanisms that may operate against adult cestodes in dogs. In number 5, 19 mature T. multiceps were expelled 147 days after infection whereas in 1 and 4 no such event was apparent. Rickard, Coman and Cannon (1977) commented that the literature on immunity to adult cestodes was both confusing and contradictory, and their own studies demonstrated that in five successive infections of dogs with Cysticercus pisiformis, they could not demonstrate any effect of the previous infections that could not be attributed to age. Gemell quoted by Rickard (1983) reported that the susceptibility of dogs to an initial infection is highly variable and is not age dependent. Some dogs for instance acquire resistance to reinfection and this is manifested by the complete rejection of the subsequent challenge. Other dogs however remained susceptible to challenge after 8 challenge infections over a two year period. This supports evidence produced by Herd (1977) who suggested that natural resistance factors operate in some dogs. Gemell and Soulsby (1968) earlier had reported that even when dogs acquire an immunity after repeated infections, this is not absolute and is easily lost.

Immunity to adult cestodes is manifested in two ways. The cestodes may die and be expelled from the intestine or may undergo destrobilisation (Hopkins, Subramanian and Stallard 1972, Gray 1972). As far as dog No 5 was concerned, the adult cestodes were expelled simultaneously but they were not dead. This suggested that some factor had operated upon them, causing them to become detached from the intestinal mucosa, and subsequently expelled alive which does not conform with the accepted "self cure" phenomenon, when worms die and are then expelled. As far as the other two dogs Nos 1 and 4 were concerned it is likely that the established cestodes underwent destrobilisation and eventually disintegrated as no evidence of their existence was found after treatment with Cestarsol.

These experimental infections demonstrated the biotic potential of T. multiceps infections even when such infections are light compared with some of the field infections (Chapters 3.4 and 3.5). Each gravid proglottid before release contains 37,000 oncospheres (Herbert and Willis 1984) thus the experimentally infected dogs would have voided an average of between 53,000 and 125,000 oncospheres daily, dispersed in the faeces and in proglottids, into the environment over a period of 80 - 240 days.

| Dog number | Number of <u>T. multiceps</u> scolices fed | Day on which proglottids first detected | Number of proglottids detected per day | | Number of days no proglottids detected | Last day on which proglottids detected | Number of <u>T. multiceps</u> recovered |
|------------------|--|---|---|---------|---|---|---|
| | | | Range | Average | | | |
| 1 | 2 | 42 | 0-7 | 1.9 | 32 | 140 | 0 |
| 2 | 5 | 51 | 0-8 | 2.1 | 16 | 180* | 2 |
| 3 | 5 | 61 | 0-4 | 1.7 | 19 | 180* | 2 |
| 4 | 10 | 47 | 0-16 | 2.8 | 8 | 121 | 0 |
| 5 | 5 | 49 | 0-11 | 3.4 | 11 | 180* | 3 |
| 5 ⁽¹⁾ | 30 | 56 | 1-21 | 4.3 | 2 | 147 | 19 |

Table 1 Rate and duration of proglottid release of T. multiceps in 5 experimentally infected dogs

(1) Reinfected at 14 months of age

* Infection terminated

4.2 Experimental infection of rabbits, mice and lambs

Certain authors, notably Clapham (1942), Esch and Self (1965), Esch (1964, 1967), postulated that T. multiceps and T. serialis should be regarded as one species. Clapham (1942) based her arguments on morphological grounds. Esch (1964, 1967) on the other hand based his arguments on a series of experimental infections of mice with T. serialis oncospheres, in which he demonstrated that coenuri of this parasite developed in the brain. He concluded that the barriers preventing successful establishment and development of the larval forms of T. serialis in an intermediate host were largely host rather than parasite orientated. He also was of the view that if this was the case, the coenuri of this cestode were distributed widely and were capable of developing in a variety of host tissues, in a wide range of intermediate hosts.

It must be emphasised however that Esch (1964, 1967) derived the oncospheres for the experimental infections in mice from cestodes which had developed from coenuri recovered from jack rabbits, cotton tail and swamp rabbits trapped in Oklahoma. No oncospheres derived from coenuri of ovine origin were included in his experimental work and

it was decided therefore to determine the host specificity of the larval stage of T. multiceps derived from an ovine source in rabbits, mice and lambs.

MATERIALS AND METHODS

T. multiceps oncospheres were obtained by harvesting from gravid segments of the cestode voided in the faeces of experimentally infected dogs. The proglottids were placed in a petri dish containing approximately 5 ml of normal saline and gently pressed to expel the oncospheres. The proglottid was then removed and the suspension of oncospheres in saline then centrifuged at 2,000 revolutions per minute for approximately 10 minutes to deposit the oncospheres, which were then resuspended in distilled water and then centrifuged once more before resuspension in distilled water, at a concentration of 2,000 oncospheres per ml.

Three New Zealand White rabbits, one male and two female, obtained from the Central Veterinary Laboratory (CVL), approximately 26 weeks of age and weighing 2.5 kilos, were lightly anaesthetised with chloroform and a 2 mm bore plastic tube passed into the oesophagus and stomach. A 5 ml hypodermic syringe was attached to the tube and 1000 oncospheres suspended in 3 ml of distilled water were administered. A further rabbit of the same breed, age and

weight, received 3 ml of distilled water only in a similar manner. All the rabbits were returned to their individual cages and fed on hay and pellets for the remainder of the experimental period.

Albino mice, 12 weeks of age and weighing approximately 35 gms were obtained from the CVL breeding colony. A total of 36 mice, 18 male and 18 female were lightly anaesthetised with chloroform. Twelve of these, 6 male and 6 females, were infected with 1000 oncospheres suspended in 0.5 ml of distilled water by means of a 1.0 ml hypodermic syringe and a specially prepared 16 BW gauge hypodermic needle passed into the oesophagus. Another twelve, 6 males and 6 females received 1500 oncospheres administered in a similar manner. A further twelve mice, 6 males, 6 females received 1.0 ml distilled water only.

Two Welsh X Speckle faced lambs, one male TM1 and one female TM2 aged five months and weighing 29 and 24 kg respectively, bred at the laboratory and reared indoors were experimentally infected one with 500 and the other with 750 oncospheres. The oncospheres were suspended in 15 ml of distilled water and the suspension administered into the lamb's oesophagus via a stomach tube. The lambs were then returned to their loose box and maintained on sheep nuts and hay for a period of three weeks, during which blood and cerebrospinal fluid samples were taken for

laboratory examination (Chapter 6.1). After three weeks they were turned out on the the paddocks for the remainder of the experiment.

Post mortem examinations were carried out on the rabbits, mice and lambs and selected tissues subjected to a histological examination.

RESULTS

1. Rabbits The three experimentally infected and control rabbits were observed for a period of 120 days, during which none showed any clinical signs of disease. Euthanasia was carried out by the intravenous injection of pentobarbitone sodium and a post mortem examination carried out. No ceonuri were found in the subcutaneous tissues, muscles, viscera or central nervous system. Sections of the brains and livers were examined histologically. No evidence of parasitic invasion or development was detected in either brains or all livers of all four rabbits.

2. Mice Four mice from each of the three groups, were killed by chloroform at 28 days post infection, when the mice in all groups appeared clinically normal. At post mortem examination there was no macroscopic evidence of parasitic invasion or development and microscopic

examination of stained sections of liver and brain confirmed the absence of lesions. After another 28 days, four mice from each group were killed and examined in a similar manner and again no evidence of parasitic invasion or development was found. The remainder of the mice were killed 96 days after infection, but no macroscopic or microscopic evidence of parasitic infection was found.

3. Lambs TM1 remained clinically normal for 150 days, when euthanasia was carried out by the intravenous injection of pentobarbitone sodium. No lesions attributable to parasitic infection were found in any of the abdominal or thoracic organs or in the carcass. A small necrotic lesion 3 mm in diameter was found on the surface of the right cerebral hemisphere at the frontal pole. The lesion was calcified and a histological examination revealed that it was of a parasitic nature (described in Chapter 6.2).

TM2 developed clinical signs of coenurosis 112 days post infection and euthanasia was carried out 28 days later. A post mortem examination revealed no significant abdominal or thoracic lesions but a typical Coenurus cerebralis cyst was identified in the right cerebral hemisphere.

DISCUSSION

In a preliminary study Esch (1964) demonstrated that T. serialis, which he considered to be T. multiceps, could be established in laboratory mice. He also found that the administration of cortisone increased the rate of infection in the subcutaneous tissues, the normal site for the location of coenuri in rabbits, but not in the central nervous system. In a later study Esch (1967) found that experimentally infected female mice and naturally infected female jack rabbits were more frequently infected than males, which he concluded was evidence that females were more susceptible to the larval form of T. serialis (T. multiceps).

In contrast a number of workers in the USSR over a period of years attempted to infect rabbits and rodents with oncospheres of T. multiceps and failed (Erchov 1961). Furthermore they investigated the possible role of rodents as intermediate hosts of T. multiceps under field conditions, but were unable to find any positive evidence.

In the present study infection was not established in either male or female mice and rabbits. Thus it seems that the one strain of T. multiceps was incapable of establishing in either of these species although one of the two lambs infected developed clinical coenurosis. This together with the observations of workers in the USSR

suggests that Esch was in error when he concluded that T. multiceps and T. serialis were the one and same species. Indeed Meyer (1955) earlier had expressed the view that there were sufficient differences in morphology and intermediate host range, between the two parasites to justify them being regarded as distinct species. Dunn (1978) on the other hand whilst accepting that the two adult parasites shared certain common morphological features, considers that there is a substantial biological difference in intermediate hosts and tissue location of larval stages between the two species, although they may have been derived from a common ancestor and that consequently may have developed as a result of host and/or parasitic adaptation. Verster (1969) in her taxonomic study of Taenia species considers that on morphological grounds the two species are distinct.

During an epidemiological survey of bovine leptospirosis at Carmarthen during 1974 - 1976, several hundred mice and rats were subjected to a post mortem examination and none were found to have any coenuri in the subcutaneous tissues or central nervous system. Out of approximately 500 rabbits trapped by the Ministry's pest control officers in the southern area of the old county of Pembroke during 1972 - 1974 only three had subcutaneous coenuri of T. serialis and the protoscoloces from one was fed to a dog and the mature cestode was identified by Mr

Prudhoe as T. serialis. Thus the coenuri of this cestode was not widely distributed in the rabbit populations of south Pembroke.

There was no doubt that the strain of T. multiceps used in this study was capable of infecting and producing clinical disease in sheep. Esch's studies would have been more meaningful if sheep had been included in his experimental studies and if a strain of T. multiceps from a clinical case of coenurosis in sheep or cattle had also been used.

It was of interest that neither lambs developed clinical signs of acute disease, despite the fact that in earlier work (unpublished evidence by the author, 1966) acute coenurosis could be produced in lambs 4 - 12 weeks of age with 500 - 1000 oncospheres. This suggested that lambs develop an immunity at about three months, which has since been confirmed by G T Edwards and I V Herbert (personal communication).

5 THE VIABILITY OF THE ONCOSPHERE AND COENURUS OF
TAENIA MULTICEPS

5.1 The viability of the oncosphere

Although the transmission of T. multiceps from the definitive to the intermediate host is direct, the oncospheres are exposed to environmental conditions over varying periods of time. Thus the influence of environmental factors on their survival is important in the epizootiology of ovine coenurosis.

Much of the work on taeniid oncosphere survival has been based on an in vitro assessment of viability, but this has not been done for T. multiceps in Great Britain. It must be emphasised however, that an in vitro assessment may not provide a true indication of the infectivity of oncospheres, and because of this the results of such assessments must be interpreted with a certain degree of caution. Whilst it is accepted that the viability of T. multiceps oncospheres is best assessed by in vivo studies, the long and costly experimental procedures required together with the practical difficulties at the laboratory made such studies impractical. It was therefore decided to study the survival of T. multiceps oncospheres by the

in vitro methods described by Silverman 1954) and Laws(1968) which are based on the hatching of the oncospheres in artificial hatching fluids.

MATERIALS AND METHODS

The oncospheres used in the study were obtained from gravid proglottids of T. multiceps shed by experimentally infected dogs. The proglottids were macerated in saline and the suspension so obtained was filtered through a sieve of 100 μ m aperture to remove as much proglottid tissue as possible, and then centrifuged at 2000 rpm for 15 minutes to deposit the oncospheres, which were then further washed in two changes of distilled water by centrifugation. A thick suspension of oncospheres in distilled water, containing approximately 500,000 oncospheres per ml was then prepared and distributed in 2.0 ml volumes in 24 x 3 ml plastic tubes. The mouth of each tube was then covered by a layer of muslin; held in place by an elastic band and stored for eight weeks under different environmental conditions. Eight of the tubes were held in a refrigerator at 4°C, eight tubes were stored in a cupboard in the laboratory at a temperature of 14 - 20°C and the remainder were buried up to the neck under cover in the soil of one of the laboratory paddocks at air temperature which varied from 3° - 11°C.

At weekly intervals one tube from each of the storage sites was removed and the oncospheres examined for viability by treatment with artificial hatching fluids, namely, artificial gastric fluid (AGF) as described by Meyers (1957) and artificial intestinal fluid (AIF) as outlined by Silverman (1954). The constituents of each of the fluids are listed below.

AGF

Pepsin 0.29 gm, $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ 0.19 gm,
NaCl 8.0 gm, 36% HCl 2.0 ml,
Distilled water to 1000 ml, pH adjusted to 1.8

AIF

Pancreatin 0.5 gm, Sodium tauroglycocholate 0.5 gm,
Bactotryptone 0.2 gm, Bactcholesterol 0.02 gm,
Distilled water 50 ml, pH adjusted to 7.8

To each of the tubes 1.0 ml of AGF was added, which were then shaken vigorously and placed in a water bath at 37°C for 30 minutes. The tubes were then removed from the water bath, centrifuged for 10 minutes at 2000 rpm to sediment the oncospheres and the supernatant fluid discarded. Two millilitres of AIF were added to the deposited oncospheres which were re-suspended in the fluid by vigorous shaking and the tubes incubated once more in the water bath for 30 minutes. Aliquots of approximately

0.25 ml were withdrawn from the tubes after 10, 20 and 30 minutes incubation and examined on a warm slide for evidence of hatching under a microscope. The number of live and dead hexacanth in a total of 100 were recorded.

RESULTS

There was a considerable amount of evaporation from the tubes held in the laboratory cupboard, so that after a three week period, all the liquid had evaporated leaving a dry deposit on the sides and bottom of each tube. This was entirely due to the consistently high temperature and low relative humidity within the laboratory at all times. Evaporation from the tubes held in the refrigerator occurred at a much lower rate, but even so, at the end of six weeks storage, these were also completely dried out. In contrast the tubes buried in the paddock did not dry out and at the end of the eight week period 1.0 ml of fluid remained.

The hatching process observed was similar to that described by other authors. The vitelline layer rapidly disintegrated, quickly followed by an expansion and darkening of the oncosphere. In turn this was followed by further expansion of the oncosphere which then became a dark brown or black colour. Finally the embryophoric

blocks were released and the hexacanth embryos liberated. Viable hexacanth embryos were recognised by their motility, whilst non viable ones were non motile.

The results obtained are summarised in Table 1.

Under conditions of higher temperature 14 - 20°C and low relative humidity, there was a sharp drop in the number of viable oncospheres, so that none were detected after the third week. When held at 4°C, viable oncospheres were identified up to the end of the fifth week only. The oncospheres held under "field" conditions were still viable at the end of the eighth week.

DISCUSSION

The results obtained from this study confirm that taeniid oncospheres can survive for varying periods under differing environmental condiditons. The longest survival period was recorded under field environmental conditions when the ambient temperatures fluctuated between 3° and 11°C, although the oncospheres were held under cover and not exposed to rainfall during this period. The oncospheres held in the laboratory cupboard and in the refrigerator were exposed to low relative humidity, which

undoubtedly was an important factor in their relatively short period of survival in comparison with that of the oncospheres stores in the soil of the paddock.

Coman (1975) demonstrated in parallel in vitro and in vivo experiments with T. pisiformis oncospheres, that temperature and relative humidity are important factors in determining the survival period. He found that a high temperature (38°C) was quickly lethal, irrespective of relative humidity and very few oncospheres survived beyond seven days. Gemell (1975) quoted by Coman (1975) obtained similar results in vitro with oncospheres of T. hydatigena and T. ovis. Coman (1975) also demonstrated that a combination of low temperature and low relative humidity reduced the survival period to approximately 56 days, whereas oncospheres stored at low temperatures and high relative humidity survived for 300 days. He concluded that T. pisiformis oncospheres could survive for more than one year under favourable environmental conditions on pasture.

The survival period recorded in the present study are broadly in line with those reported by Coman (1975) and others, and clearly demonstrates the effect of low relative humidity. It must be emphasised however that these results do not necessarily reflect the infectivity of the oncospheres. It is possible that some of the

oncospheres which hatched in the artificial hatching fluids would not have been infective in in vivo experiments; although Coman (1975) demonstrated a close agreement between the results of his in vivo and in vitro experiments. Williams and Colli (1970) showed however that the activation of E. granulosus oncospheres in artificial hatching fluids was not an accurate indication of infectivity since hexacanth embryos which did not activate were still infective for laboratory rodents. Thus it is possible too that in the present study oncospheres which did not hatch in the artificial hatching fluid were infective for sheep.

As far as T. multiceps is concerned it is likely from information obtained in the present study that oncospheres can survive for a period of weeks or months under weather conditions prevailing in Great Britain. During winter months, when the ambient temperatures are low and relative humidity high, oncospheres are likely to remain viable for much longer periods. Abassov (1965) during a series of grazing experiments in Russia, demonstrated that T. multiceps oncospheres remained viable for two months when air temperatures fluctuated between -16° and 10°C and Erchov (1961) found that oncospheres covered by snow for 160 days were still viable.

Willis and Herbert (1984) investigated the effect of cold and heat on oncospheres in a similar manner to that undertaken in the present study. Oncospheres frozen at -20°C for up to 42 days were still capable of hatching. At temperature between -20°C and below 37°C , oncospheres remained viable for 42 days, but at 37°C , hatchability had been lost within 7 days. Eggs held out of doors and subjected to fluctuating May and June temperatures, but protected from direct sunlight lost their ability to hatch within 7 days.

When T. multiceps oncospheres are deposited on pasture either in proglottids or dispersed in dog faeces, factors other than temperatures and relative humidity must be taken into consideration of the epizootiology of coenurosis. These include the consistency and rate of breakdown of the faeces, effects of rainfall and ultraviolet light and the rate of grass growth. Proglottids rapidly putrify and thus the oncospheres would have been more quickly released than from faeces. Oncospheres in liquid faeces are more easily washed out on to the pasture than from solid faeces which may take three weeks or more to break down. Coman (1975) found that rain washed large numbers of T. pisiformis oncospheres into soil, but even after a cumulative rainfall total of 300 mm, sufficient remained on the pasture to infect rabbits grazing experimental plots. The average annual rainfall

in the old county of Carmarthen recorded at the Carmarthen Veterinary Investigation Centre between 1971 and 1976 was 1250 - 1500 mm, but in certain parts of the county, the upper Towi and Cothi valleys for example, the annual average was 10 - 20% higher. Although this may have resulted in a smaller number of oncospheres being retained on the sward, the high rainfall would have induced a more rapid growth of the sward, so that both factors would have diluted the infection on the sward. A more critical study of these factors would supplement the basic information provided by the in vitro assessment of the viability of the oncospheres.

| Storage temp. (C) | Number of live/dead oncospheres | | | | | | | |
|----------------------|---------------------------------|-------|-------|-------|-------|-------|-------|-------|
| | Wk 1 | Wk 2 | Wk 3 | Wk 4 | Wk 5 | Wk 6 | Wk 7 | Wk 8 |
| At 4° | 72/28 | 63/37 | 52/48 | 37/63 | 30/90 | 0/100 | NE | NE |
| At 3-11° | 74/27 | 70/30 | 64/36 | 57/43 | 41/59 | 35/65 | 22/78 | 11/89 |
| At 14-20° | 61/39 | 29/71 | 4/96 | 0/100 | 0/100 | 0/100 | NE | NE |

Table 1 The survival of T. multiceps oncospheres under three laboratory conditions, assessed by an in vitro method

5.2 The viability of the coenurus

The transmission of T. multiceps from the intermediate to the definitive host, is not only dependent on the availability of the larval stage Coenurus cerebralis in the intermediate host, but also on the ability of the larval stage to remain viable and infective until such time as the definitive host consumes the coenurus. There is only one report in the literature on the viability of the coenurus of T. multiceps (Abassov, 1965). In a series of experimental studies he demonstrated that the coenurus remained viable for a few days only. In view of the dearth of information available it was decided to study the viability of coenuri at the Carmarthen laboratory.

MATERIALS AND MEHTODS

Four "worm free" Border Collie cross puppies aged between four and five months of age were obtained for the experimental work. Coenuri were derived from four sheep affected with chronic coenurosis and fed to the puppies.

The sheep were killed by the intravenous injection of pentobarbitone sodium and the heads immediately removed from the carcasses. Two of the heads were held at 4°C in a refrigerator, one for three days and the other for six days. The other two heads were exposed to environmental

conditions on the laboratory paddock, but protected from predators by a metal mesh meat cover, one for three days when the ambient temperature ranged from 11° - 14°C and the other for six days at an ambient temperature of 11° - 17°C. No rainfall was recorded during either of the two periods.

After the appropriate period the heads were skinned and the brain exposed by the removal of the cranial roof. The coenuri were located, identified and the state of preservation noted. Twenty scolices were removed from each, incorporated into a small ball of lard or canned meat and then fed to a puppy, which was then confined in a loose box for six weeks and fed on canned meat and biscuits or biscuit meal. After six weeks the puppies were treated with Cestarsol and the purged faeces examined for the presence of T. multiceps as described in Chapter 3.4.

RESULTS

Inevitably the storage of ovine post mortem material leads to autolytic and putrefactive changes. The two heads stored at 4°C showed little putrefaction compared with the two heads held at ambient temperature. The brains of the refrigerated heads were firm and each contained a single coenurus. The wall of these coenuri were translucent and

the cyst fluid clear. In contrast, the brains, kept at ambient temperature, were soft and the brain tissue pink due to blood staining . Each contained a single coenurus, which had opaque walls, turbid foul smelling cyst fluid, especially the one held for six days.

The results of feeding 20 scolices to each of four puppies and subsequent treatment with Cestarsol are summarised in Table 1.

The 20 scolices from the coenuri derived from the heads held in a refrigerator at 4°C for three and six days infected the puppies with 12 or 15 mature specimens of T. multiceps respectively. In contrast, only 4 mature specimens were recovered after infection by scolices derived from coenuri stored at ambient temperature for 3 days and none from the puppy which had received scolices from the brain exposed for six days.

DISCUSSION

This experimental study, although limited in extent and not controlled provides results of some interest. The putrefaction in the head tissues and brains held at 9 - 17°C were dramatic and to be expected. Most putrefactive organisms in ovine carcasses originate from the gastro-intestinal tract and removal of the head prevented

their massive invasion. Even so in both heads the coenuri had been affected by putrefaction which would be more severe in field conditions particularly during the summer. It must also be remembered that wool is an effective heat insulator which would prevent rapid heat loss and thereby assist bacterial invasion, multiplication and putrefaction.

The survival of larval cestodes have been studied in vitro by inducing protoscolices to evaginate (Smyth 1967). Anderson and Loveless (1978) for example used this method for E. granulosus cysts and concluded that under moderate spring or fall conditions in North America, the larval stage could survive for one or two weeks, but during the hot summer months, the survival period would be reduced to one or two days. However it must be emphasised that these authors studied the survival of the protoscolices after removal of the cysts from organs such as liver and lungs, and therefore such a study cannot be fairly compared with a study of survival in a decomposing carcass. Furthermore it is by no means certain that the evagination of protoscolices in vitro reflects their ability to infect the definitive host.

Willis and Herbert (1984) studied the survival of a single coenurus in a sheep's head after death. The head was removed from the carcass and placed in an incubator at 10°C for seven days. The coenurus was then removed from

the brain and protoscolices treated by the method described by Smyth (1967). All the protoscolices were active despite the fact that the brain showed considerable signs of decomposition. They concluded that the coenurus can survive in a decomposing brain for several days and probably longer. However they did not assess whether the protoscolices which evaginated after 7 days were still infective.

Abassov (1965) demonstrated that coenuri of T. multiceps removed from sheep heads and held at -3 - 19°C for one, three, five and ten days failed to infect puppies. Thus the coenurus has a similar resistance to freezing temperatures, as Cysticercus bovis and Cysticercus cellulosae, the cause of measly beef and pork. Thus, freezing temperatures are used to render affected carcasses fit for human consumption. Abassov (1965) also found that coenuri exposed to sunshine for one day were viable and infective for dogs, but not after for three days. In another study coenuri buried in the ground for two days remained viable and infective, but if they remained buried for five days they failed to infect young dogs. All these coenuri had been removed from the brain and therefore were not subjected to the usual post mortem changes which sheep carcasses undergo.

The results from the present study, those by Abassov (1965) and Willis and Herbert (1984) provide similar results on the survival of coenuri of T. multiceps. It is evident that the viability of the coenurus is determined by a) the time which has elapsed after death of the intermediate host and b) the degree and rate of putrefaction before ingestion by the definitive host. Under field conditions it is unlikely that even under the most favourable conditions that coenuri remain viable and infective for more than a few days in Great Britain.

| Coenurus exposed for | Number of scolices fed to each puppy | Number of <u>T. multiceps</u> recovered after treat- ment with Cestarsol |
|-------------------------|---|--|
| 3 days at 4°C | 20 | 12 |
| 6 days at 4°C | 20 | 15 |
| 3 days at 9-14°C | 20 | 4 |
| 6 days at 11-17°C | 20 | 0 |

Table 1 Experimental infection of 4 puppies with scolices from coenuri stored at various temperatures

There are few published reports of experimental studies on the pathogenesis of coenurosis. Gallego (1930) experimentally infected 15 sheep and studied the pathological lesions in the brains of those that developed clinical disease. Ronzhina and Borodulina (1956) published their observations on 17 experimentally infected sheep and 2 goats which were also infected experimentally. A comprehensive account of a field outbreak of coenurosis in a sheep flock, in which the brains of 73/83 animals examined had lesions of coenurosis was reported by Fankhauser, Hinterman and Valette (1959).

A study of the pathogenesis of the disease in sheep was therefore considered desirable. Lambs were experimentally infected and observations made on the clinical signs, clinical pathology, immunology and pathology of the disease. Additional information was obtained from field outbreaks of coenurosis and data from selected field case histories are included in this section.

6.1 Clinical signs and clinical pathology

(a) Experimental infection

MATERIALS AND METHODS

Two five month old Welsh x Speckle face lambs, one male (TM1) and one female (TM2), weighing 29 and 24 kilos respectively, bred at the laboratory and reared indoors were used for the first part of the study. They had been weaned at twelve weeks of age, some eight weeks prior to experimental infection. For the second part of the study four other lambs of the same breed (TM3, TM4, TM5, TM6), two males and two females, weighing 15 - 19 kilos were selected. These lambs had also been bred at the laboratory, reared indoors from birth and weaned at about nine weeks of age, seven days prior to being experimentally infected.

TM1 and TM2 were fed on sheep nuts and hay after weaning and for three weeks following experimental infection, after which they were turned out on to the laboratory paddocks, but kept under close observation each day. The other four lambs were kept indoors for the whole of the experimental period and fed on hay and sheep nuts.

The lambs were clinically examined each day for seven days before and for up to 21 days after experimental infection and a record was kept of the daily rectal temperature. Both eyes of each lamb were carefully examined with an ophthalmoscope on at least two occasions before they were infected and daily for up to 21 days after infection. Whenever possible photographs of the retinal changes in the eyes were taken with a Kowa fundus camera.

Blood samples were taken for haematological examination from the jugular vein on the day before the lambs were experimentally infected, on the day they were infected and thereafter every other day until 21 days post infection or until euthanasia was carried out. The blood sample was taken into a 5 ml vacutainer containing 1.0 mg disodium ethylenediamine-tetracetic acid (EDTA) as an anticoagulant. In view of previous experiences of experimental infections of T. multiceps in sheep, only total red cell and white cell counts, followed by a white cell differential count was made.

A sample of cerebro-spinal fluid (CSF) was also taken from each lamb two or three days before experimental infection and again 7 and 14 days after infection. The wool over the lumbo-sacral area was clipped and the lumbo-sacral junction located by digital palpation and marked with an indelible marker. The skin over the area was thoroughly

washed and disinfected with surgical spirit and the lambs were sedated with 0.4 - 0.5 ml of a 2 per cent solution of xylazine hydrochloride (Rompun Bayer Ltd). After sedation the lambs were placed in a sitting position and a 75 mm spinal puncture needle with a stylette was introduced gently into the space between the dorsal processes of the last lumbar and first sacral vertebrae. When the needle was judged to have entered the spinal canal, the stylette was removed and fluid usually escaped. A syringe was attached to the needle and 0.5 - 1.0 ml of CSF was withdrawn. If the CSF was blood stained it was discarded and a second sample collected. A total white cell count and a differential cell count was then carried out.

The lambs were infected with oncospheres harvested from gravid proglottids of T. multiceps recovered from the faeces of experimentally infected dogs. TM1, TM3 and TM5 received 500 oncospheres, TM2 and TM4 received 750 oncospheres, TM6 received 1000 oncospheres, suspended in 15 ml of distilled water administered via a stomach tube inserted into the upper oesophagus.

(b) Field infection

Data from 6 typical field cases (FC1 - FC6) was also recorded. These cases were submitted to the laboratory by farmers at the request of practising veterinary surgeons for diagnostic purposes. All the affected animals were clinically examined; blood and CSF samples were collected as described in the previous section. Euthanasia was usually carried out within 24 hours of submission. In one case however a Coenurus cerebralis cyst was surgically removed from a nine month old female at the request of the owner and was kept under observation over a four month period following the operation.

RESULTS

(a) Experimental infection

Five of the experimentally infected lambs developed coenurosis, but no clinical signs were observed in the remaining lamb. The course of the disease and clinical signs are summarised in Table 1.

Details of the rectal temperatures, the total white cell counts and eosinophil counts for each lamb are presented in Figs 1 to 6.

TM1 This lamb remained clinically normal throughout the 150 day period after infection when euthanasia was carried out. The rectal temperature, total white cell and eosinophil counts remained within the normal range during the 21 day period when the animal was kept indoors. No cells were detected in the CSF and no eye changes were detected by ophthalmoscope.

TM2 Pyrexia was evident on the third and fourth days after infection and again on the eleventh and twelfth days, thereafter returning to the normal range. There was a slight leucocytosis on the third and fourth day after infection, and a marked eosinophilia which persisted until the sixth day. No cells were detected in the CSF on day 7 post infection but a week later there was a total of 10 cells per cmm of which 3 were eosinophils.

TM3 There was a marked pyrexia on the second and third day and from the tenth day post infection which persisted until euthanasia was carried out. The total white cell count was elevated on the second and third day and then returned to the normal range. There was a marked increase in the number of circulating eosinophils on the second

day, but the count remained high until euthanasia was carried out. A total of 480 cells per cmm were counted in the CSF on the seventh day, which had risen to 2400 per cmm on the fourteenth day of which 67% were eosinophils.

TM4 A transient pyrexia was recorded on the second day and the rectal temperature was markedly elevated from the twelfth day. A leucocytosis and eosinophilia was detected on the second day, the counts thereafter returning to normal. Cells were detected in the CSF on the fourteenth day, when the count had risen to 1800 per cmm, of which 75% were eosinophils.

TM5 The rectal temperature was elevated on day 3 and from day 12. A slight leucocytosis was evident from day 3 - 6, but at this time there was a marked eosinophilia. The CSF sample collected on day 7 was contaminated with blood and discarded, but the sample taken seven days later gave a cell count of 2100 per cmm, 50% of them eosinophils.

TM6 There was a pyrexia between day 3 and 7 and again from day 10. A leucocytosis and eosinophilia was recorded between day 4 and 6. No cells were detected in the CSF on day 7, but on day 14 the cell count was 2600 per cmm, 75% of which were eosinophils.

(b) Field infection

The clinical signs together with a brief history of the six field cases are set out in Table 2. All cases were confirmed as coenurosis.

DISCUSSION

The experimental infections in lambs demonstrated quite clearly that acute and chronic coenurosis can be produced by relatively small numbers of infective oncospheres. Edwards and Herbert (1982b) in their studies confirmed this, although the numbers of infective oncospheres administered to their experimental sheep were 2 - 4 times greater than the numbers used in the present study.

It was not clear why the same number of infective oncospheres produced acute disease in one lamb and chronic disease in another or as in the case of TM1 no clinical disease. A number of factors must have operated, including the infectivity of the oncospheres, the resistance mechanisms of the lambs and the numbers of oncospheres which reached the brain.

The first clinical signs observed in the experimentally infected lambs were detected on or about the third day, although these were not nervous signs. They coincided

with a transient pyrexia, leucocytosis and eosinophilia, which persisted for about 48 hours. The clinical signs were of abdominal discomfort and almost certainly would have been missed had they not been under close observation. Edwards and Herbert (1982b) make no reference to such observations in the report on their experimental infections.

The first signs of nervous involvement were seen between 9 and 12 days post infection. Edwards and Herbert (1982b) recorded nervous signs appearing between 9 and 33 days post infection. Bondareva (1955) in contrast recorded the first signs of nervous involvement between 17 and 22 days post infection. The appearance and severity of the nervous signs would be largely governed by the number of larvae reaching the brain and from post mortem evidence in the present study it was evident that a significant number of larvae were "lost" in extraneural sites.

The clinical signs recorded for the acute disease both in the experimental and field infections varied from animal to animal and must have reflected the number of migrating larvae and their locations. Pyrexia and profound depression were accompanied by neurological disorder, which included ataxia, blindness, opisthotonus and recumbency. A feature in some of the acute cases was the presence of small retinal haemorrhages detected by

ophthalmoscopy. These were first reported by Ronzhina and Borodulina (1956), but have also been reported by Edwards and Herbert (1982b). The precise cause of such lesions is not known, but it is likely that they represented damage caused by migrating larvae.

Of particular interest was the demonstration of cells in the CSF in acute cases between 7 and 14 days after infection. The collection and examination of CSF is not regarded as a standard diagnostic procedure in ovine medicine, but it is a technique which in the author's view can be readily adopted in the investigation of nervous diseases. The presence of large numbers of cells, a high proportion of which were eosinophils was a constant feature of acute coenurosis and therefore should be considered pathognomonic. High CSF cell counts are also a feature of bacterial infections of the nervous system, but in contrast to coenurosis the preponderance of cells are polymorphs and lymphocytes.

The chronic disease manifests itself weeks or months after the larvae reach the central nervous system. From the evidence collected by Edwards and Herbert (1982b), chronic disease develops when relatively few larvae reach the brain and only one or two coenuri develop. In more than 80 per cent of chronic cases encountered by the author over a period of more than twenty years, only one coenurus

had developed in the brain and in the majority of the remainder there were two coenuri only. In the present study few larvae if any could have reached the brains of TM1, and those that did so must have rapidly died without producing clinical signs. The fact that no clinical signs were observed in TM2 until 112 days after infection when it developed chronic coenurosis lent support to the view that only a few larvae reach the brain of animals which are affected with chronic coenurosis. However from time to time individual farmers have reported that animals with chronic coenurosis had shown signs of "star gazing" for a few days, some weeks or months previously. The author has observed such clinical signs in a number of flocks on a number of occasions and the majority, but not all of the affected animals subsequently developed chronic coenurosis.

The nervous signs of chronic coenurosis are varied and are largely governed by the site of the developing coenurus. Interpretation of the clinical signs of any nervous disease is dependent upon a knowledge of neuro-anatomy and neuro-pathology, and an evaluation of the case history. Skerritt and Stallbaumer (1984) concluded that interpretation of clinical signs remained the best method of diagnosis, a view with which the present author agrees. These authors outline a method of neurological examination which is almost exactly the same as that which the present

author has used for a number of years. Such an examination is time consuming and not readily undertaken under farm conditions and therefore the search for diagnostic aids which can assist the practising veterinary surgeon continues.

The examination of eyes (Fig 7) for evidence of papilloedema in chronic coenurosis is a useful diagnostic technique. Whilst papilloedema may not be evident in all cases of chronic coenurosis, it is present in a significant proportion, a fact which was confirmed by Edwards and Herbert (1982b). Its detection however is not easy and may require a long experience of ophthalmoscopy before it can be regularly recognised. The author was fortunate in having had the use of a fundus camera for a period of weeks, when normal and abnormal eyes could be photographed and the prints studied at length to familiarise himself with the changes.

Although no cells could be detected in the CSF of the chronic cases examined in the present series Doherty, Bassett, Breathnach, Monagham and McErlean (1989) demonstrated a high cell count and a high proportion of eosinophils in the CSF of a sheep with chronic coenurosis. There is a need therefore to examine CSF in chronic cases to determine whether the examination of CSF can assist diagnosis.

Tirgari, Howard and Boargob (1987) reported on the successful use of contrast radiography for the diagnosis of chronic coenurosis, especially in the location of coenuri. Doherty, McAllister and Healy (1989) reported as to the value of ultrasound in diagnosis of chronic coenurosis. Both these techniques however are not readily applied by practising veterinary surgeons.

Skerritt and Stallbaumer (1984), Tirgari, Howard and Boargob (1987) and Doherty, McAllister and Healy (1989) are of the view that a high proportion of chronic coenurosis cases respond to surgical treatment. Even so it should be emphasised that a successful outcome can only be achieved if the coenurus can be located with precision and removed without causing undue brain damage.

| Lamb No | Infective dose (No of onco- spheres) | Time of onset and duration of clinical signs (days post infection) |
|---------|--|---|
| TM1 | 500 | No clinical signs (150), euthanasia |
| TM2 | 750 | (3-4) Pyrexia (11-12) slight pyrexia (112) ataxia and papilloedema (Fig 7), progressed to circling movements, blindness and recumbency (140), euthanasia |
| TM3 | 500 | (2-3) pyrexia and slight depression and abdominal discomfort (9) pyrexia depression, restlessness progressed to ataxia, blindness, recumbency opisthotonus (19), euthanasia |
| TM4 | 750 | (2-3) pyrexia, abdominal discomfort (12), marked depression, pyrexia (16), blindness, continuous aimless wandering (18), recumbent, euthanasia |
| TM5 | 500 | (3-4) pyrexia (12), marked depression, pyrexia, teeth grinding (19), severe ataxia, blindness, retinal haemorrhages, euthanasia |
| TM6 | 1000 | (2-4) pyrexia, abdominal discomfort (10), pyrexia, elevation of the head (star gazing) (11) recumbent, tetanic spasms, euthanasia |

Table 1 Time of onset and duration of clinical signs in 6 lambs experimentally infected with T. multiceps oncospheres

| Case No | Age | History | Clinical Signs |
|---------|--------|---|---|
| FC1 | 9 mths | Seem to be slightly in-coordinated on hind limbs about 6 wks before submission. Progressed over next 4 wks to posterior paralysis, since then moving on its front limbs and dragging its hind legs. No other similar cases. No tick problems. Occasional cases of coenurosis | Alert, in dog sitting position. Posture of head and front limbs normal. Reflexes normal. No sign of joint or muscle lesions. No evidence of spinal injury. Hind limb pedal reflexes exaggerated, muscles atrophied. Possible diagnosis-spinal abscess or coenurosis, but no leucocytosis, a slow onset indicative of coenurosis. Unable to collect CSF sample due to possible obstruction of spinal canal |
| FC2 | 9 wks | 8 other lambs with nervous signs had been lost over a period of 7-10 days prior to submission of lamb. Another 10-12 lambs affected. Louping ill had been diagnosed in flock previously but ewes and lambs since vaccinated, including the affected group. No cases of coenurosis previously recorded | In comatose condition. Pupils of both eyes widely dilated. Few small haemorrhages on retina of both eyes. No pedal reflexes. Subnormal rectal temperature. No sign of traumatic injury and no tick infestation. CSF - cells 4,000 cmm 82% eosinophils confirming acute coenurosis |
| FC3 | 7 wks | Animal had been "circling" for about one week prior to submission. Coenurosis had been diagnosed previously in the flock but not for a period of 4-5 years. Two other animals of similar age had been slaughtered a few weeks previously because they were thought to be "giddy", | Animal circles in an anti-clockwise direction only. Blind in the right eye, which showed evidence of papilloedema (Fig 7a). Frontal bone over left cerebral hemisphere "springy" under digital pressure. No blood or CSF abnormalities. Typical chronic coenurosis |

but brains not examined

| | | | |
|-----|------|---|--|
| FC4 | 1 yr | Three animals in a group of 40 females overwintered on a dairy farm were showing nervous signs when they returned to the home farm. Cases of coenurosis had occurred in the flock regularly. Animal submitted had deteriorated very rapidly over a period of about 1 week | Abnormal posture of head and neck, head held high and neck extended. Slight muscular tremors of neck. "Straddle legged" posture reluctant to move, unco-ordinate. No evidence of blindness and no papilloedema. No bone rarefaction and no leucocytosis and no cells in CSF. Signs indicative of cere-brallar coenurosis |
|-----|------|---|--|

| | | | |
|-----|--------|--|--|
| FC5 | 9 mths | Periodic signs of in-coordination which usually persisted for 2 or 3 days. Over the 7 days prior to sub-mission signs had become continuous and the animal was now very excitable. Coen-urosis had not been confirmed in the flock but was known to exist in the neighbouring flocks | Animal very bright but hypersensitive to external stimuli. Head deviated to the left, blind in left eye, sluggish papillary reflex and evidence of papilloedema (Fig 7b). Capable of moving in clockwise and anticlockwise direction. Frontal bones over right cerebral hemisphere yielding to digital pressure. No blood or CSF abnormalities. Typical chronic coenurosis. At farmers request a 30 ml cyst removed surgically from the right cerebral hemisphere. The residual cavity was still apparent post mortem 4 months later (Figs 2 d & e; 6.2) |
|-----|--------|--|--|

| | | | |
|-----|--------|---|--|
| FC6 | 6 mths | Two six months old live lambs submitted from a flock in which 9 had died over the previous two weeks. Although coenurosis had been confirmed in the flock, neither the owner or his veterinary surgeon considered it to be the cause of | One lamb had died soon after submission. Other lambs recumbent, but when placed on sternum unable to maintain normal head and neck posture. Pedal reflexes normal. Normal rectal temperature, slight leucocytosis. No blindness. Signs inconclusive, but other |
|-----|--------|---|--|

death. Louping ill had not been confirmed in the flock but was now suspected by the veterinary surgeon lamb had evidence of acute coenurosis

Table 3 Case histories and clinical signs of six selected field cases of coenurosis in lambs

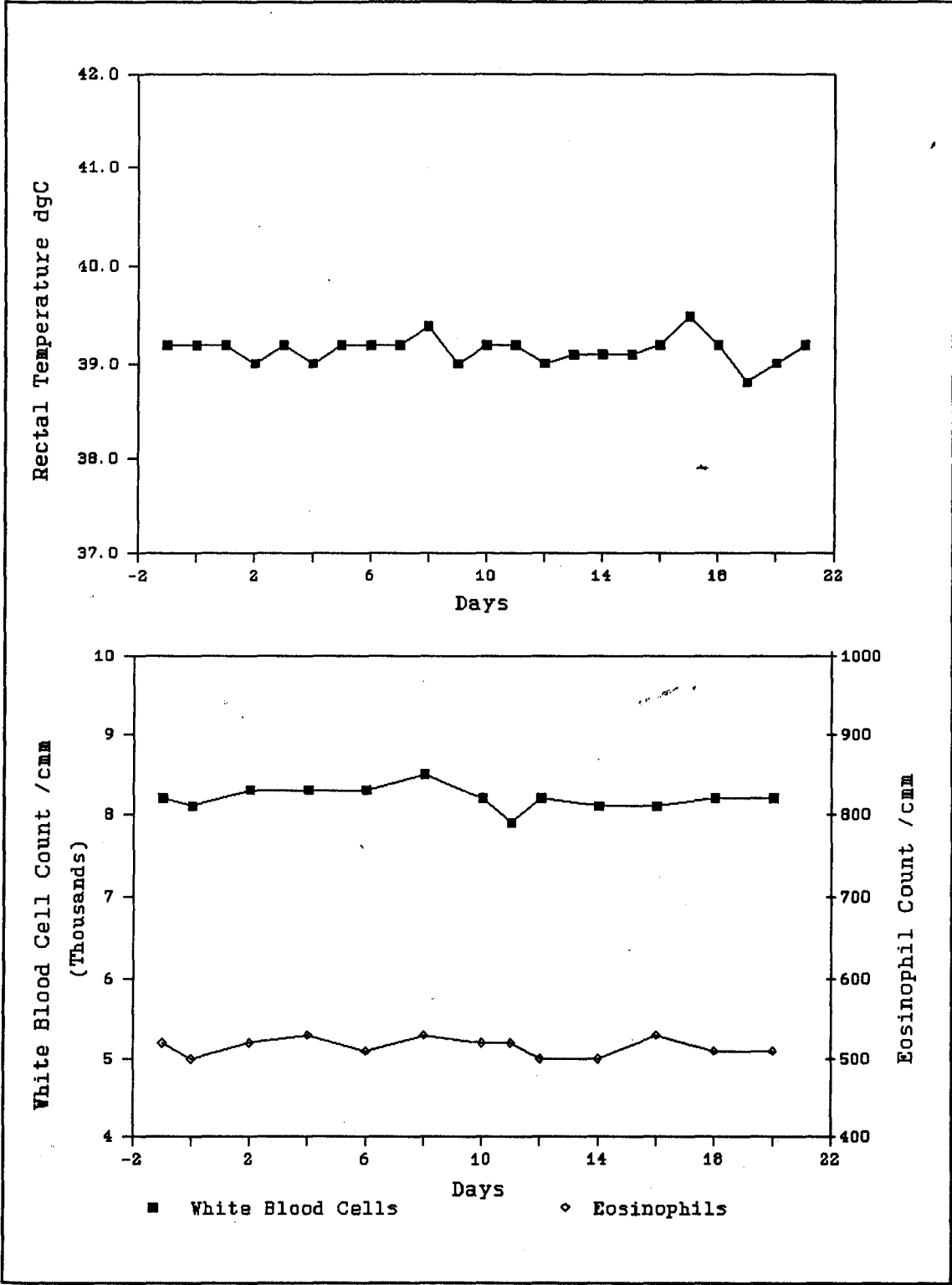


Figure 1 : Records of rectal temperature, total blood white cell and eosinophil counts for TM1.

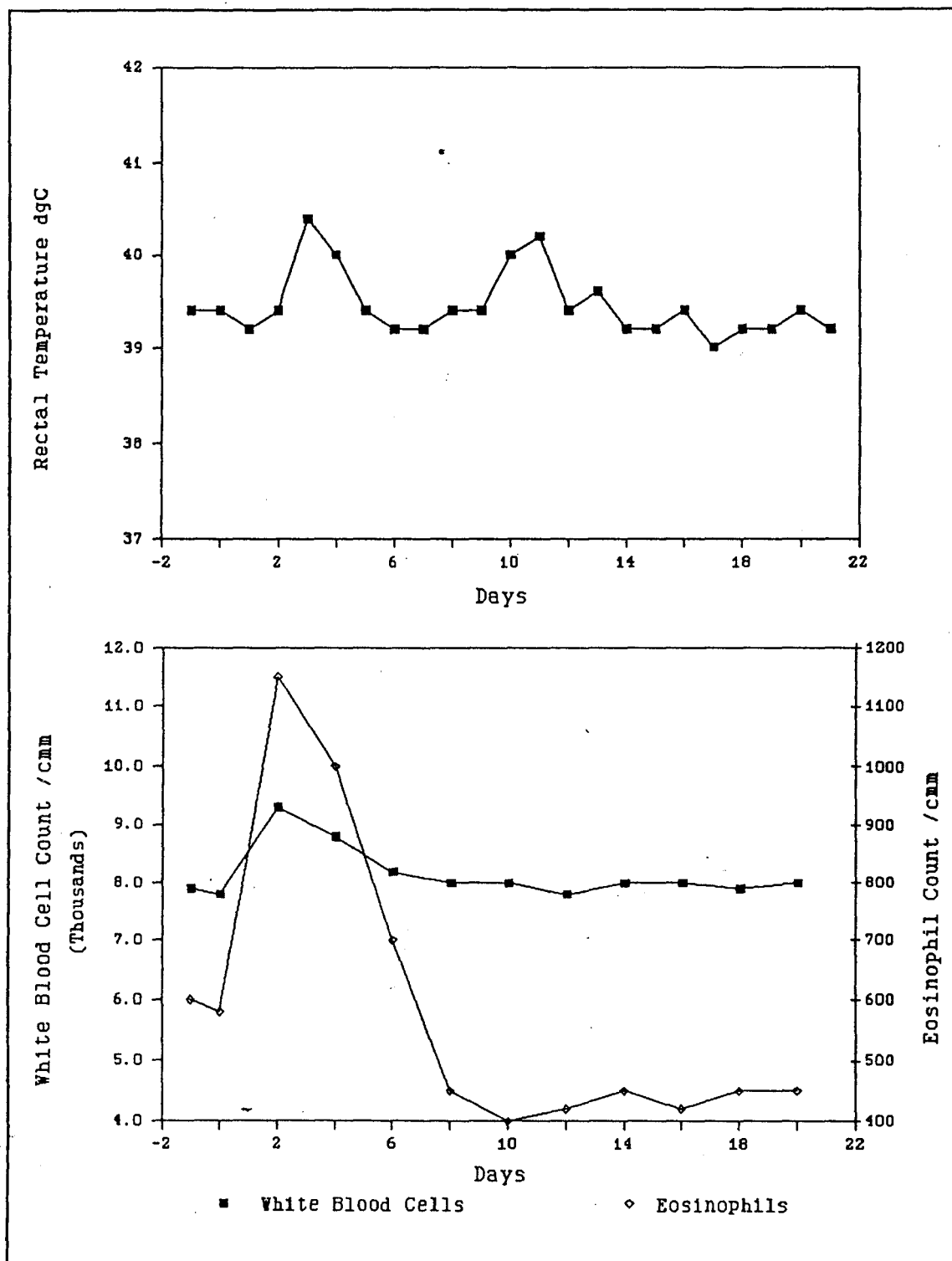


Figure 2 Records of rectal temperature, total blood white cell and eosinophil counts for TM2.

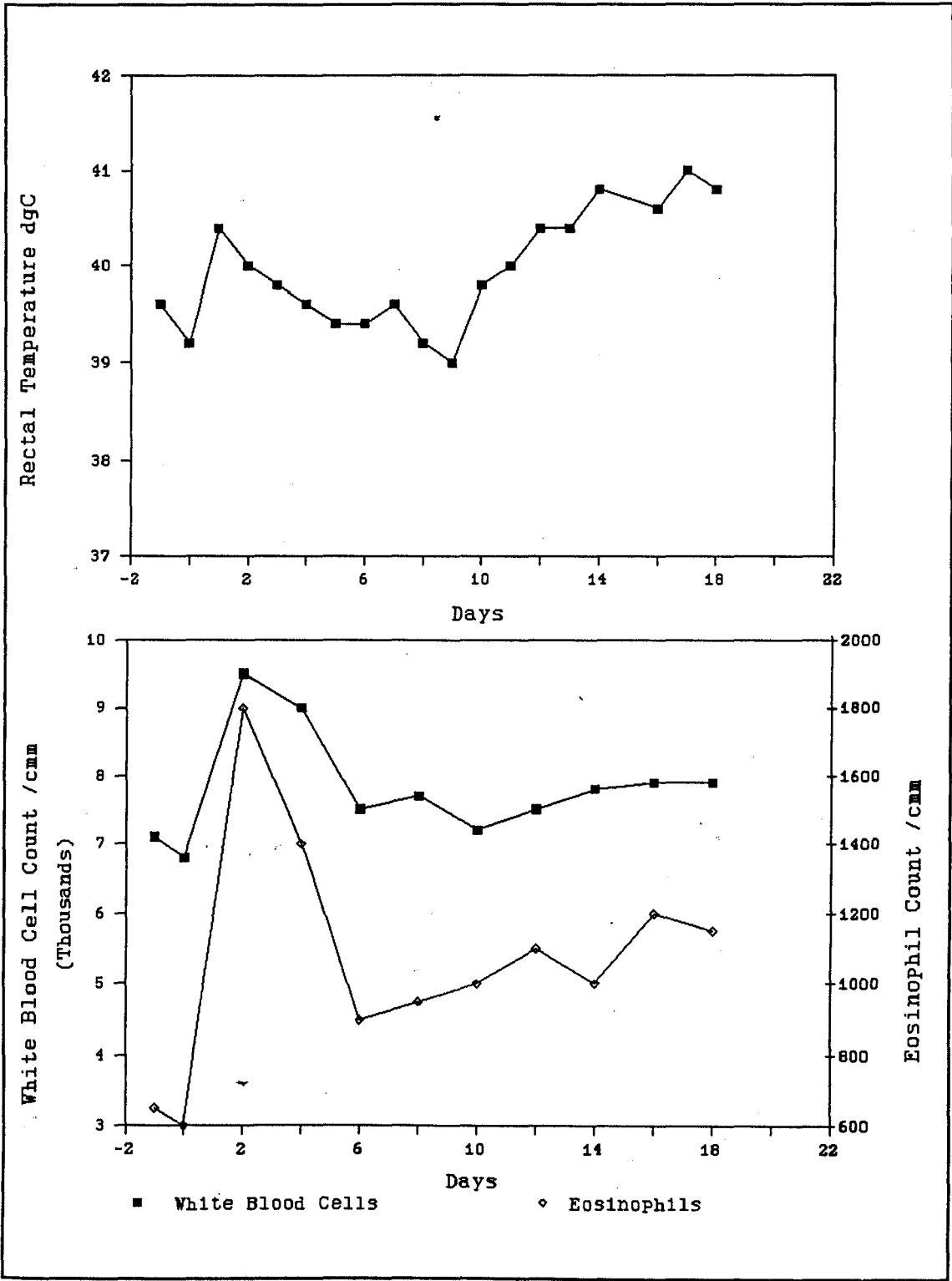


Figure 3: Records of rectal temperature, total blood white cell and eosinophil counts for TM3.

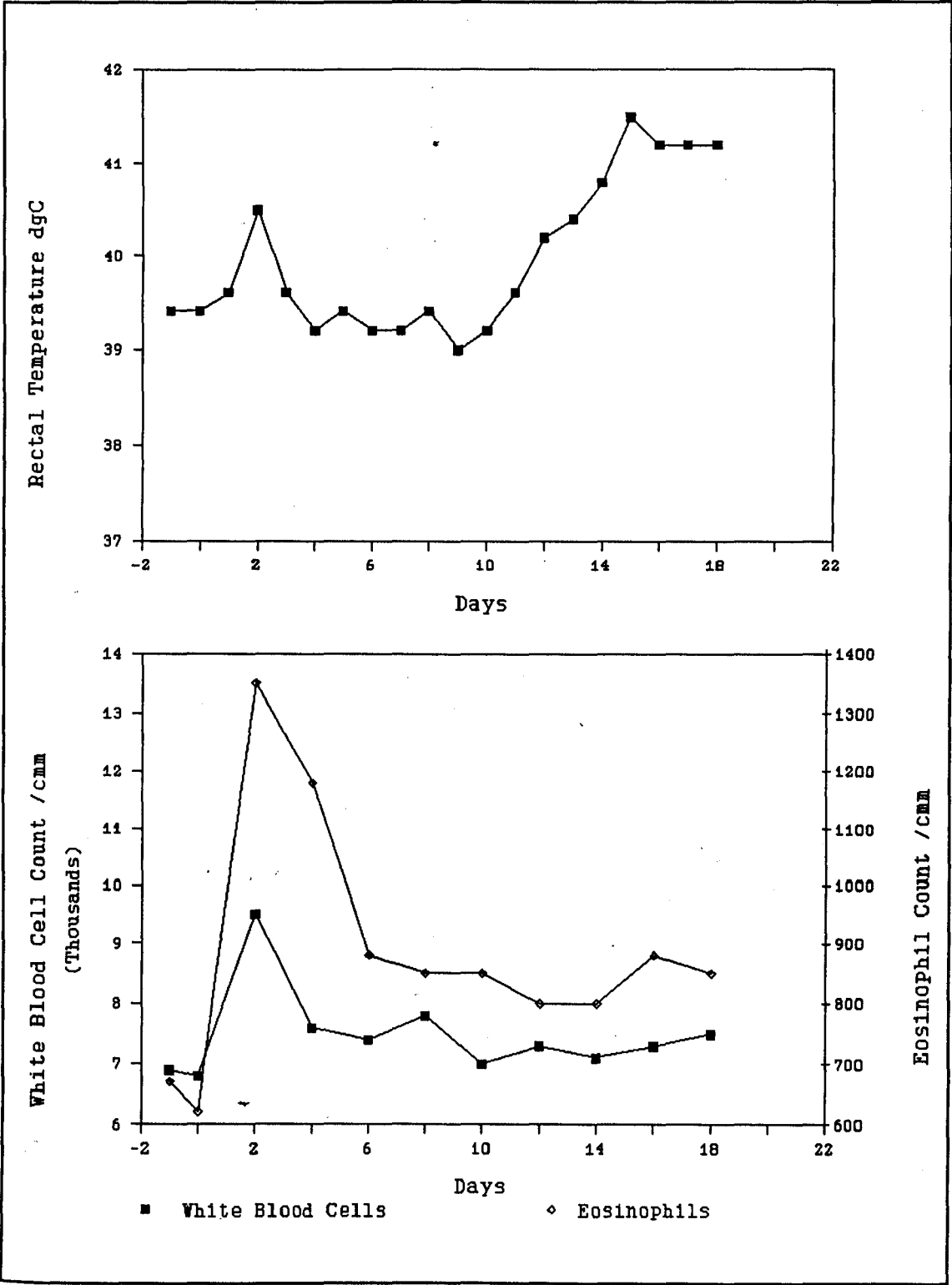


Figure 4: Records of rectal temperature, total blood white cell and eosinophil counts for TM4.

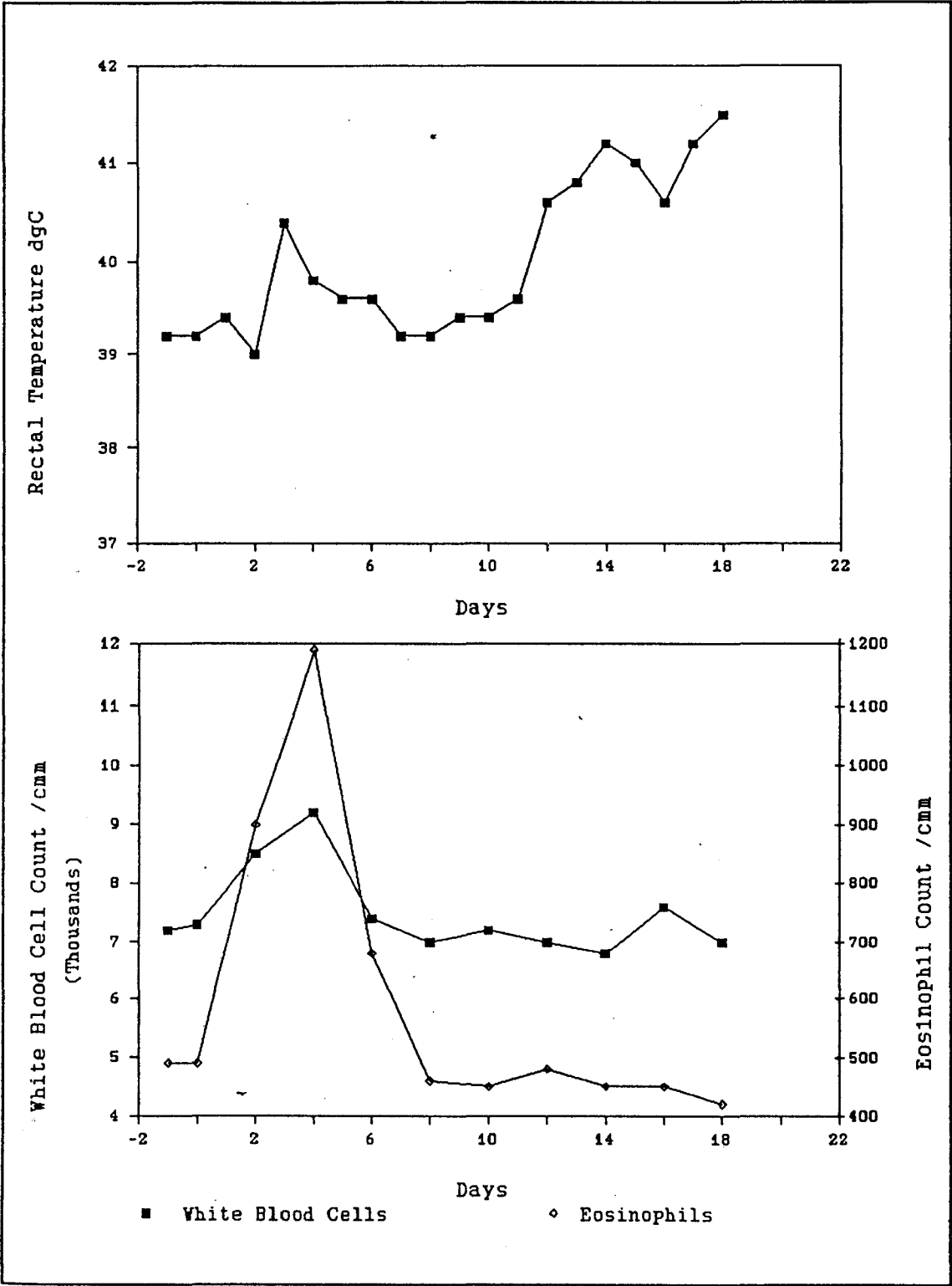


Figure 5 : Records of rectal temperature, total blood white cell and eosinophil counts for TM5.

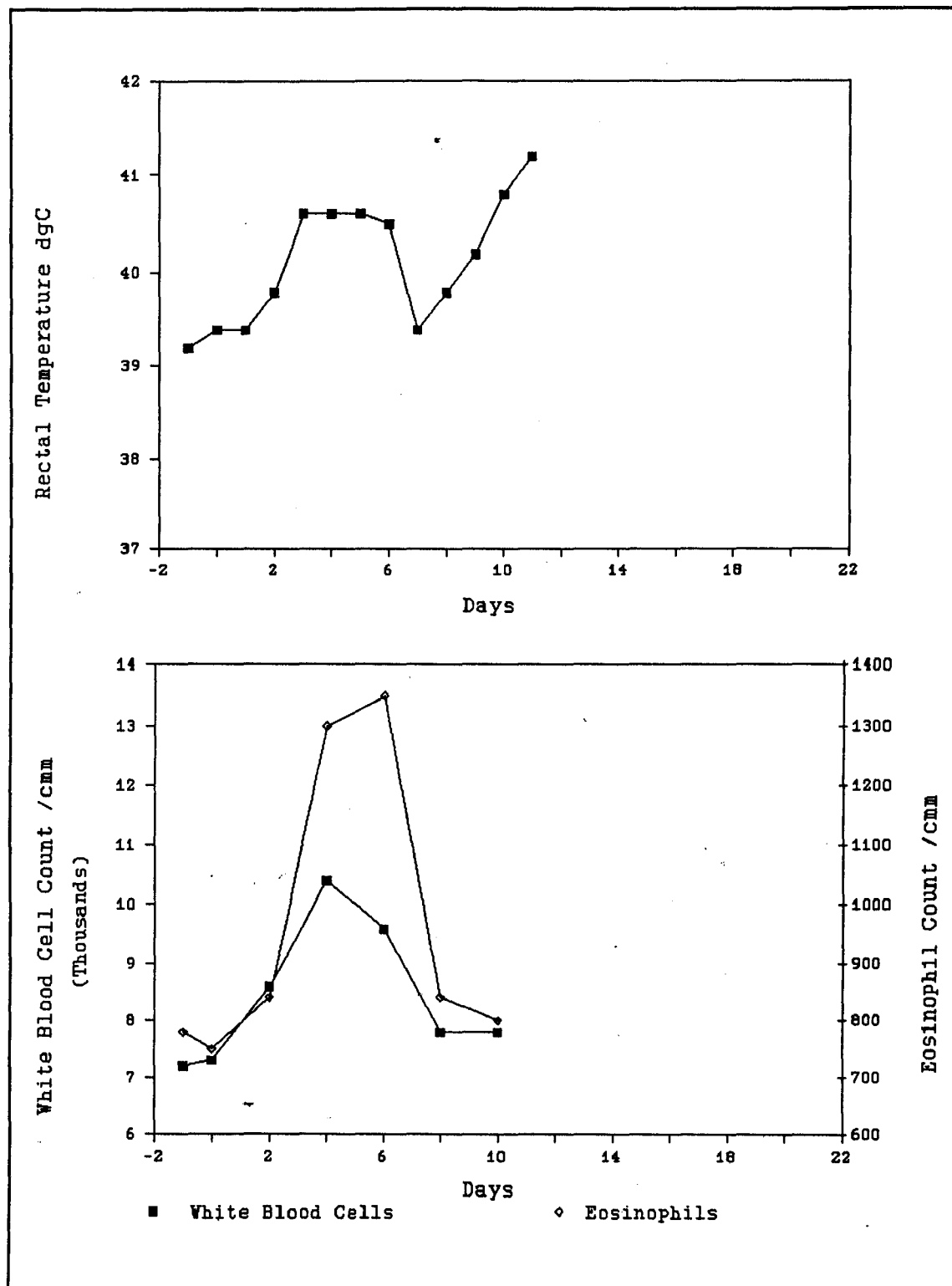


Figure 6: Records of rectal temperature, total blood white cell and eosinophil counts for TM6.

Fig 7 **Papilloedema in chronic coenurosis**

a) **In lamb Fc3 (Table 3)**

b) **In lamb Fc5 (Table 3). In both cases the outline of the optic disc is indistinct and in Fc5 the main retinal vessels are also affected**



6.2 Pathology

In the previous section the clinical signs and clinical pathology of experimental and naturally occurring coenurosis were described. An account of the gross and microscopical pathology is presented in this section and the findings discussed in relation to the published reports of other authors.

MATERIALS AND METHODS

The affected sheep (TM1 - 6 and Fc1 - 6 as in 6.1) were killed by the intravenous administration of pentobarbitone sodium and the carcasses bled out by severing the carotid arteries and jugular veins. A detailed post mortem examination was immediately carried out and all the thoracic and abdominal organs including the gastro-intestinal tract, were carefully examined for evidence of parasitic invasion and development. The brains and spinal cords were exposed in a standard manner and after careful visual examination were removed for histological examination.

The brains and when appropriate, the spinal cords, were immediately immersed in approximately ten times their volume of 10% buffered formol saline for a period of at least ten days until complete fixation. Each brain was

then coronally sectioned with a sharp ham knife at intervals of approximately 3 - 4 mms. Blocks of tissue were then prepared from representative areas of the cerebrum, cerebellum and the brain stem, including the medulla oblongata, and embedded in paraffin wax. Sections were cut from each block and stained with haematoxylin and eosin, van Giseon or Romanes stains as described in the Manual of Veterinary Investigation Laboratory Techniques (Reference Book 366).

Suspect lesions from thoracic and abdominal organs were also subjected to histological examination. Blocks of tissue in the form of 2 - 3 cm cubes were taken from the parenchymatous organs and heart and fixed in buffered formol saline. Suspect lesions in the intestine were also subjected to microscopical examination, affected portions were first washed in saline and then fixed in formol saline. The fixed tissues were then trimmed, embedded in paraffin wax, sectioned, stained with haematoxylin and eosin and when considered necessary with van Gieson's, Masson's trichrome or Gordon and Sweet's methods as described in the Manual of Veterinary Investigation Laboratory Techniques (RVG6).

RESULTS

(a) Gross Pathology

(i) Experimental Group

TM1 No clinical signs of coenurosis had been observed in this animal during the whole of the experimental period. It had continued to thrive and during the post mortem examination it was found to be in very good body condition, the unopened carcase weighing 49.5 kilos. There were no obvious macroscopical lesions in any of the abdominal or thoracic organs attributable to parasitic invasion and development, but there were a few small areas of consolidation in the apical lobe of the right lung.

A small circumscribed lesion was found on the surface of the right cerebral hemisphere, but there were no other brain or spinal cord lesions visible to the naked eye.

TM2 During the 28 days this animal had shown clinical signs of chronic coenurosis it had failed to thrive and this was reflected in the weight of the unopened carcase which was 37 kilos. There were only sparse fat deposits around the kidneys and in the omentum. No lesions were detected in the abdominal or thoracic organs, although

some bruising of the subcutaneous tissues were obvious over the thorax and abdomen which had occurred during recumbency.

The frontal bones over both cerebral hemispheres were extremely thin and were easily penetrated with a knife. On exposure, both cerebral hemispheres were found to be very turgid with flattened convolutions. A large coenurus cyst, in excess of 50 ml in volume, occupied most of the right cerebral hemisphere, dorsal to the lateral ventricle but extending posteriorly and occluding the third ventricle and the cerebral aqueduct, thereby obstructing the drainage system of the left lateral ventricle and causing its distension. On removal of the coenurus, both cerebral hemispheres collapsed (Fig 1e).

TM3 - 6 These four lambs developed signs of acute coenurosis and the lesions found at post mortem examination were similar and therefore the following description of the gross pathological lesions applies to all.

Because of the relatively short course of the disease, body condition of the lambs was good and the carcase weights were similar to those at the commencement of the

experiment. However there was some evidence of dehydration and bruising of the subcutaneous tissues of TM3, 4 and 6 following periods of recumbency.

The parenchymatous organs of the lambs were rather dark due to congestion and in one lamb, TM6, a small amount of clear fluid had accumulated in the pericaardial sac, thoracic and abdominal cavities. Small yellowish necrotic areas were evident on the epicardium of both ventricles and in TM3 these were found deeper in the myocardium (Fig 1b). A few small yellowish areas were also found in the lungs and on the surface of the kidneys of this lamb including a number of tortuous yellowish tracks (Fig 1a). A close examination of the small intestine revealed a number of yellowish green tracks, 5 - 10 mm in length, in the mucosa of the duodenum and jejunum, which in some places appeared to have extended through to the serosa. The associated mesenteric lymph nodes were moderately enlarged and oedematous, and when incised a small number of small yellowish green foci were detected.

The macroscopic lesions in the brain were very obvious and indeed spectacular. There was an excess of turbid cerebrospinal fluid. A varying number, but more than 20, yellowish white tracks of varying length extended over the surface of the cerebral hemispheres and around the base of the brain (Fig 1c & d), especially in the vicinity of the

optic chiasma. In the pituitary fossa there were purulent deposits. After fixation and when the brain was coronally sectioned necrotic areas, purulent deposits and tracks became apparent in the depths of the sulci and there were purulent deposits in the lateral ventricles. In two of the lambs yellowish necrotic areas were apparent in the basal ganglia. In only one of the lambs were macroscopic lesions evident in the cerebellum but there were none in the medulla oblongata or spinal cord.

(ii) Field cases

Fc1 The body condition was poor with marked muscular atrophy of the hind limbs. The subcutaneous tissues of the ventral abdomen and hind limbs were extensively bruised and oedematous, but there were no significant lesions in the abdominal or thoracic cavity.

Examination of the brain revealed no obvious lesions, but the spinal cord within the canal of the first three lumbar vertebrae was obviously enlarged. Closer examination revealed it to be distended from within, there being only a thin shell of nervous tissue covering a coenurus cyst (Fig 2a).

Fc2 The body condition of this lamb was good, but there was extensive bruising of the subcutaneous tissues of the thorax and hind limbs. There were no apparent thoracic lesions apart from a hypostatic pneumonia of the right lung, nor were there any obvious lesions in the abdominal cavity, including the gastrointestinal tract.

Evidence of acute coenurosis was found in the brain in the form of a small number of yellowish tracks on the surface of both cerebral hemispheres (Fig 2b). A large yellowish necrotic area was found in the region of the basal ganglia on sectioning with a ham knife.

Fc3 This animal was killed in a fair condition. There were no thoracic or abdominal lesions except for a calcified lesion approximately 1.5 cm in diameter, on the anterior surface of the liver, possibly the result of parasitic invasion.

The frontal bones over the right cerebral hemisphere were markedly rarefied and the hemisphere turgid, with flattened convolutions, and an obvious atrophy of the cortex. A large (50 ml) cyst was located in the hemispheres occupying most of the posterior half.

Fc4 This animal's body condition was extremely poor. There were no obvious lesions in any of the abdominal or thoracic organs. However there was a marked rarefaction of the bones over the right cerebral hemisphere and the hemisphere was turgid with almost a complete loss of sulci over its posterior half, due to increased pressure from within (Fig 2c). The cerebral cortex consisted of a thin shell over a cyst of approximately 60 ml. The increased intracranial pressure had also affected the cerebellum, the vermis extending posteriorly and protruding through the foramen magnum.

Fc5 This ewe had succumbed to metritis four months after a coenurus cyst had been surgically removed and two weeks after it had given birth to a single lamb. The only abdominal and thoracic lesions observed were those associated with a metritis.

An examination of the operation site revealed complete healing of the skin incision and of the trephined area of the skull which was marked by a thickening of the periosteum. The right cerebral hemisphere was obviously larger in volume than the left with some of the gyri flattened, especially anteriorly and medially (Fig 2d & e). On palpation this hemisphere was more turgid than the other and when cut transversally a large fluid filled

cavity was located dorsal to the lateral ventricle, compressing the left hemisphere and its ventricle (Fig 2e).

Fc6 Both lambs submitted were examined although only one was alive on arrival at the laboratory. There was extensive bruising of the subcutaneous tissues over the abdomen and thorax and the carcasses showed evidence of dehydration. A few small yellowish necrotic areas were found in the lungs and kidneys and in one lamb a number of yellowish tracks were identified in the mucosa of the duodenum.

Yellowish tracks were found on the surface of the cerebral hemispheres of both lambs and in the depths of the sulci. In one lamb a developing coenurus, approximately 5 mm in diameter, was found in the substance of the cortex near the median extremity of the hemisphere, and in addition several areas of necrosis were identified in this hemisphere and in the other (Fig 3) when coronally sectioned.

(b) Histopathology

For descriptive purposes histopathology is described under two headings:-

- (i) acute coenurosis which include the findings in the experimental animals TM3, 4, 5, 6 and in the field cases Fc2 and Fc6
- (ii) chronic coenurosis which covers the description of the lesions in the experimental animals TM1, TM2 and the field cases Fc1, Fc3, Fc4 and Fc5.

(i) Acute coenurosis One of the features of the acute disease was the presence of lesions in organs other than the central nervous system.

The intestinal lesions were generally healing, but even so there was clear evidence of tracking from the mucosa into the submucosa and sometimes extending as far as the serosa. The mucosal epithelium had been shed and in some areas the tips of the villi had been lost. The mucosa and submucosa were infiltrated by leucocytes, a high proportion of which were eosinophils (Fig 4f). There was much evidence of vascular destruction with haemorrhage into the submucosa. In other areas there was a proliferation of connective tissue with thickening of the intestinal wall. The associated lymph nodes were markedly oedematous and in some areas the nodes completely lost

normal architecture. In other areas there were several circumscribed areas of necrosis surrounded by an area of cell infiltration. These lesions were usually well delineated from the surrounding area by a proliferation of fibrous tissue and a cellular zone of epithelioid cells, leucocytes, predominantly eosinophils, and occasional giant cells. The necrotic tissue was often infiltrated particularly at the margins by leucocytes mainly lymphocytes and eosinophils, many of which were degenerating (Fig 4a & b).

The lesions in the kidneys were confined to the cortex and mainly located just under the capsule. They consisted essentially of a well circumscribed central area of necrotic tissue surrounded by a zone of leucocytes and fibrous tissue. Eosinophils were prominent in this zone, but there were few giant cells. The necrotic core was often infiltrated by leucocytes, a high proportion of which were degenerating (Fig 4c - e). Liver lesions were also subcapsular and resembled those in the kidneys.

Myocardial lesions closely resembled those in the kidneys and liver, with necrosis of the muscle fibres and infiltration by leucocytes, mainly eosinophils, and lymphocytes. Lung lesions when present and especially in TM3, were prominent as well defined modular necrotic areas in the apical and cardiac lobes. They consisted of a

central necrotic area surrounded by a dense zone of leucocytes infiltration with some connective tissue proliferation. More remote from the lesion there was a proliferation of the alveolar cells and both arterioles and bronchioles were surrounded by prominent "cuffs" of eosinophils and lymphocytes.

The brain lesions in all the acute cases could only be described as spectacular. In stained transverse sections of the cerebral hemispheres, lesions could be identified by the naked eye both in the meninges, and in the cerebral cortex where small cores of liquefied tissue had been lost in preparation (Fig 5a - c). There were large numbers of tracks in the meninges especially in the depths of the sulci and often in the cortex and medulla. The route of the migrating parasite was marked by a zone of loose reticular tissue in which plasma and red cells were evident. In the cerebral cortex the route of migration was marked by a central zone of liquefactive necrosis with small haemorrhages into the area. Many blood vessels around the area showed endothelial proliferation, whilst in others thrombi had formed. Around the tracks giant cells, leucocytes, both neutrophils and eosinophils formed a well defined barrier. Further away from the tracks there were well defined areas of necrosis in the cerebral cortex, similar to those in polio-encephalomalacia and in

some sections these could be associated with vascular damage in the form of thrombosis or occlusion of vessels by tissue damage in adjacent areas.

In areas of the brain not directly associated with migrating larvae some of the meningeal vessels showed endothelial proliferation, whilst in others there was a proliferation of the adventitia.

In serial sections it was possible to follow the track of migrating larvae from the lumen of a small blood vessel through the brain tissue. The tissue appeared to have undergone liquefaction to leave cavities, often crossed by a few reticular fibres, and surrounded by a zone of leucocytes (Fig 6a - e). A high percentage of the leucocytes surrounding the liquefied tissue had pyknotic nuclei and were clearly degenerating, whilst in more distant areas the predominant cell types were lymphocytes and eosinophils. Often two or more tracks became confluent with the result that a large area of the hemisphere had become necrotic (Fig 6a). There was no evidence of microglial infiltration in any of the sections, but occasionally small nests of Gitter cells were identified in areas which appeared to be recovering from the damage inflicted by the migrating parasites. In some areas necrotic tissue was infiltrated by small numbers of leucocytes, and as such areas were not directly

associated with parasitic migration, it was assumed that these lesions had resulted from anoxia caused by loss of blood supply through thrombosis (Fig 6e). Although active oncospheres were not identified in the lesions, degenerating forms, especially hook fragments were sometimes seen.

Lesions in other parts of the brain were uncommon and seldom was the cerebellum subjected to parasite invasion. Lymphocytic and eosinophilic infiltration of the meninges were the main lesions encountered (Fig 6d), although in one lamb TM3, two migratory tracks were identified. The characteristics of the lesions were similar to those described in the cerebrum.

Lesions were found in the brain stem of two lambs. These were essentially the same as those seen in the cerebrum, with the tracks extending from the meninges to the region of the corpora quadrigemina. Although the direct damage resulting from migration appeared to be limited, secondary necrosis due to anoxia was much greater than in the cerebral hemisphere. As in the cerebrum there was no evidence of microglial infiltration.

(ii) Chronic coenurosis The main and most significant feature of this phase of the disease was the absence of acute inflammatory lesions. There was no evidence of any

permanent damage caused by T. multiceps in any of the abdominal or thoracic organs of sheep affected with chronic coenurosis apart from Fc3, which had a calcified lesion on the liver.

The lesions found in the cerebrum of TM1, which did not develop clinical signs of coenurosis merits a brief description. This animal had been experimentally infected and euthanasia was carried out 150 days post infection. The suspect lesion in the cerebrum was examined microscopically and found to be necrotic with a calcified centre surrounded by reticular fibres in which there was a small number of leucocytes and eosinophils. In the calcified tissue a structure which closely resembled the handle of a cestode rostellar hook was identified.

Particular reference must also be made to the absence of active inflammatory changes in the brain of Fc5 and the area of the cerebral cortex through which the coenurus had been removed, had healed. The cerebral cortex although reduced in thickness in certain parts showed no significant changes and there was no evidence of vascular cuffing in the meningeal vessels.

In the brains from the remaining animals, the pathological changes were characteristic. It appeared that the inflammatory changes had decreased as the coenurus

increased. The cellular reaction consisted almost entirely of eosinophilic infiltration. Around the coenurus there was a narrow band of fibrous connective tissue, which in the longer standing cases resembled a limiting membrane. In the proximity of the cyst the meninges were infiltrated by small numbers of eosinophils and blood vessels in the meninges were surrounded by "cuffs" of eosinophils. The cerebral cortex surrounding the coenurus showed atrophy, in that the layers of cells were much reduced and similar changes were seen in the hemisphere not directly affected, when the intracerebral pressure had increased. In some cases the cerebellum was affected by increased intracranial pressure and as a result atrophy was apparent in the loss of cells in both granular and molecular layers of the cortex and there was some round cell infiltration of the meninges.

In the case of spinal coenurosis (Fc1), the pathological changes in the spinal cord resembled those in the brain of chronic coenurosis cases. Essentially this was atrophy of the spinal cord, with only a thin shell of nervous tissue surrounding the coenurus. There was a complete loss of the central gray matter and most of the nerve tracks forming the white matter had been lost due to pressure atrophy. The meninges were thickened and infiltrated by round cells and small numbers of eosinophils.

DISCUSSION

Innes and Saunders (1962) cite only three reports on the pathology of coenurosis, namely Gallego (1930), Singer (193) and Fankhauser, Hinterman and Valette (1959). Another report by Ronzhina and Borodulina (1956) has attracted very little attention. Of these reports, that by Fankhauser, Hinterman and Valette (1959) is the most comprehensive, in which the brain lesions encountered in a field outbreak of coenurosis in sheep in Switzerland are described. In none of the reports however is there a reference to lesions in organs other than the central nervous system.

The presence of lesions in abdominal and thoracic organs of animals affected with acute coenurosis was not unexpected as such animals were killed or died within a period of 12 - 21 days of infection. Even so there was evidence that some intestinal lesions at this stage were healing and this may account for the absence of descriptions of intestinal lesions in reports by others. The lesions were confined to the duodenum and jejunum, confirming that in common with other cestodes, the hexacanth of T. multiceps hatch and penetrate the mucosa at these sites. Whilst it appeared that most larvae had entered the blood vessels at these two sites, others had been transported to the mesenteric lymph nodes and had either left this location or been destroyed there.

The presence of lesions in the liver, lungs, kidneys and myocardium demonstrated that the hexacanth had been widely disseminated and caused necrosis and marked cellular reaction at these locations but there was no evidence that the parasite had become established at these sites. Lange (1983) described similar lesions in lambs experimentally infected with T. multiceps oncospheres, but in addition recorded parasitic invasion of skeletal muscles. He found that resolution of these lesions resulted in either calcification or focal accumulation of lymphocytes and that no larvae developed into metacestodes at these extra neural sites. Whilst this may be true in a wider sense, Baxter (1958) and I Thomas (personal communication) identified coenuri of T. multiceps in the subcutaneous tissues of a sheep and cow respectively.

The acute brain lesions in the experimental infection and in the field cases studied were similar to those described by Fankhauser, Hinterman and Valette (1959). The tracks were predominantly on the surface of the cerebral hemispheres and in the depths of the sulci and this together with the characteristics of the lesion indicated that the larvae had emerged from the smaller blood vessels. It was not possible to accurately estimate the number of larvae that had reached the brain, but it would

appear that there were between 50 and 75 individual tracks in the brain tissue of the experimental lambs, approximately 10% of the oncospheres administered.

In the experimental acute disease and in field cases of acute coenurosis the cerebrum was the site of the vast majority of lesions. It was not evident why this should be so, but Edwards and Herbert (1982b) in their experimental work demonstrated that 89% of the lesions were in the cerebrum. It is more than likely that the distribution of blood vessels in different areas of the brain accounts for the distribution of the lesions.

The damage caused by the hexacanth could not be entirely attributed to the direct physical effects of migration. Liquefaction of brain tissue and cavity formation suggested that the hexacanth produced enzymes or possibly metabolites which facilitated migration. However some necrotic areas were remote from migrating parasites and were undoubtedly associated with thrombus formation in some arterioles which may have been directly or indirectly damaged by the migrating parasite. It is likely therefore that at least some of the damage caused in acute coenurosis was not attributable to the parasite, but followed the loss of blood supply.

Chronic coenurosis was characterised by the absence of acute inflammatory changes as reported by Gallego (1930), Singer (1931), Ronzhina and Borodulina (1956) and Fankhauser, Hinterman and Valette (1959). The development of the coenurus is a gradual process and the increase in volume is accompanied by an increased intracranial pressure. Thus the atrophy induced by such changes is also gradual and this was reflected in the brains examined. There was a gradual reduction in the thickness of nervous tissue overlying the coenurus with eosinophilic cuffing of blood vessels and round cell infiltration as the only cellular reaction. Rarefaction of the bone overlying the distended cerebrum was not accompanied by any inflammatory changes in the periosteum.

The surgical removal of coenuri continues to be practised by farmers and veterinary surgeons and improved techniques result in a higher proportion of recovery. Skerritt and Stallbaumer (1985) claim that the recovery rate may be as high as 80 per cent. It has always been assumed that the damage caused by the coenurus and surgical interference is small when animals make a complete recovery. However the permanent damage in the brain of Fc5 demonstrated that even when the damage to the cerebrum is not inconsiderable, animals can compensate for the loss and make a complete recovery. In this particular case the animal made a complete recovery within 10 days of the

surgical removal of the coenurus and between that time and when it developed metritis it did not show any nervous signs.

In summary it was concluded that the development of acute clinical coenurosis was largely dependent on the number of hexacanth reaching the brain, a view which is shared by Edwards and Herbert (1982b). Acute coenurosis is infrequently diagnosed in the field and it is likely therefore that only rarely sheep, especially lambs ingest sufficient number of oncospheres to cause this form of the disease. It is possible that this occurs when whole proglottids are ingested rather than oncospheres dispersed more extensively over pasture. Such an explanation could explain why the chronic form of coenurosis is more common.

Fig 1 The gross pathological lesions in experimental coenurosis in lambs

- a) Yellowish tracks on the surface of the kidney and lung (arrowheads) in TM3 (x 1)
- b) Yellowish necrotic areas on the myocardium (arrowhead) in TM3 (x 1)
- c) and d) Yellowish tracks (arrowheads) on the surface of the cerebral hemispheres in TM3 and TM4 (x 0.67)
- e) The collapsed brain and the coenurosus in TM2 (x 0.4)

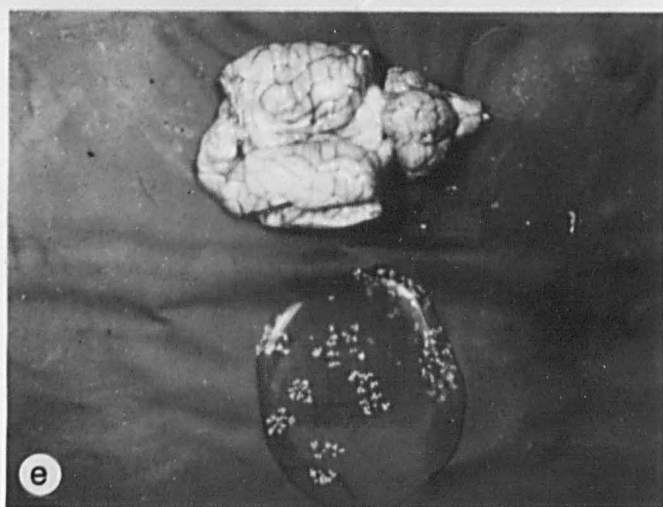
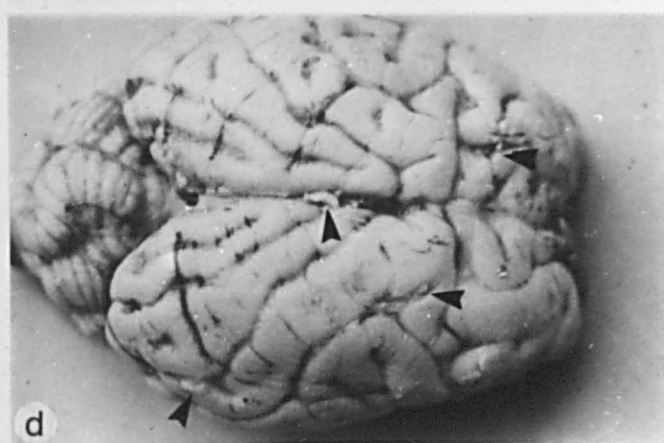
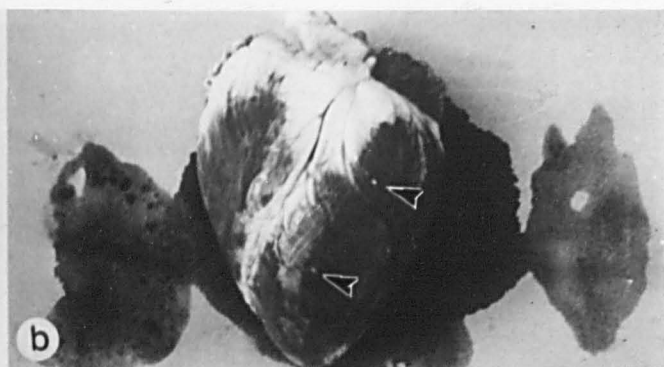
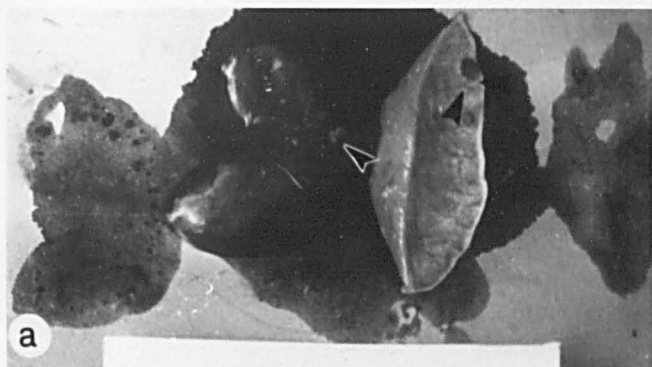


Fig 2 Gross pathological lesions in field cases of ovine coenurosis

- a) The distended spinal cord after the removal of the coenurus (Fc1) (x 2)
- b) Tracks (arrow) on the surface of the right cerebral hemisphere (Fc2) (x 0.67)
- c) Loss of sulci and turgidity of the right cerebral hemisphere and distorsion of the cerebellar vermis in chronic coenurosis (Fc4) (x 1)
- d) and e) The abnormal appearance of the right cerebral hemisphere and the residual cavity dorsal to the lateral ventricle of the same hemisphere, four months after the surgical removal of a coenurus (Fe5) (x 1) (x 1.5)

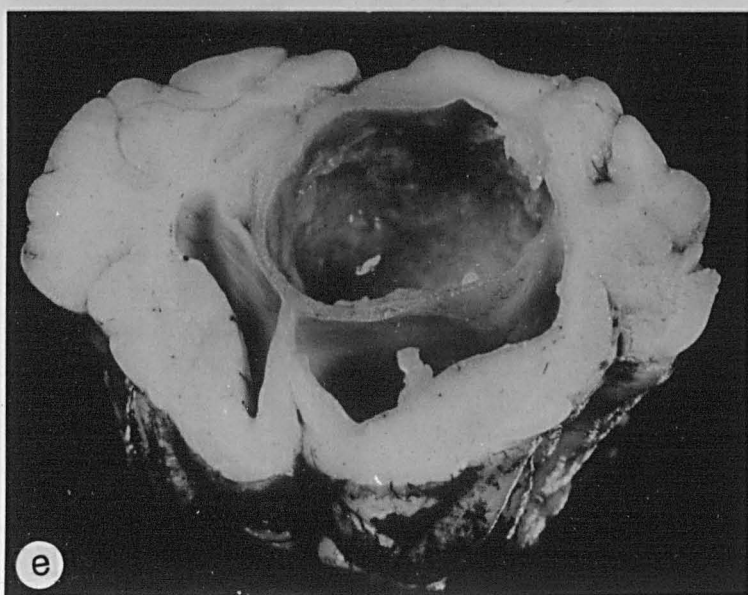
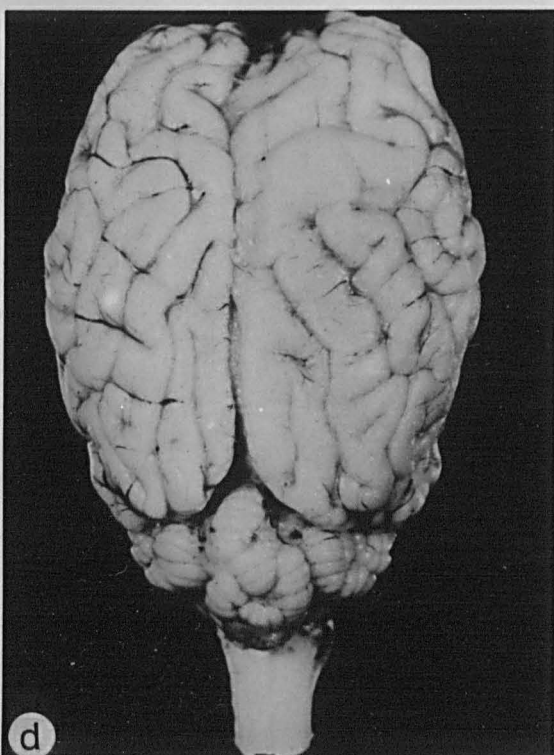
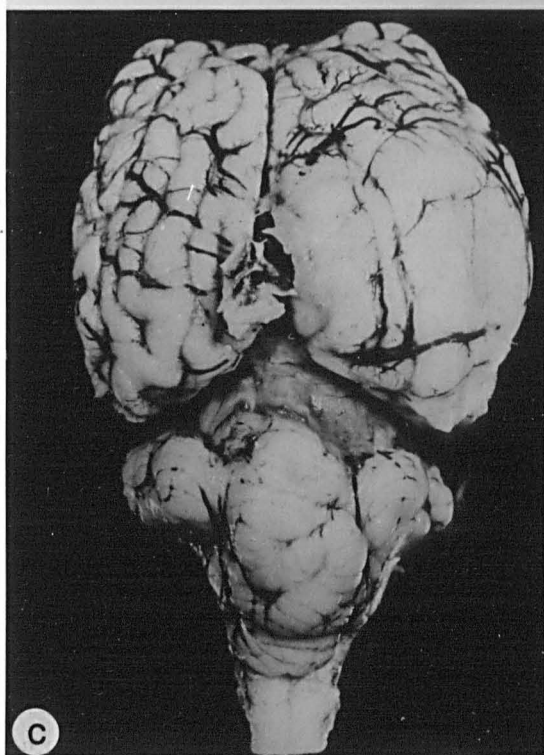
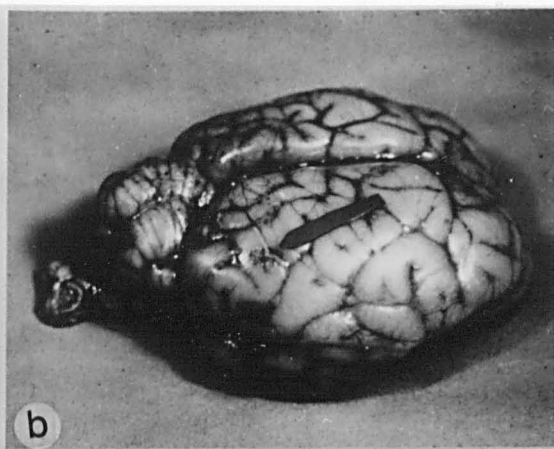
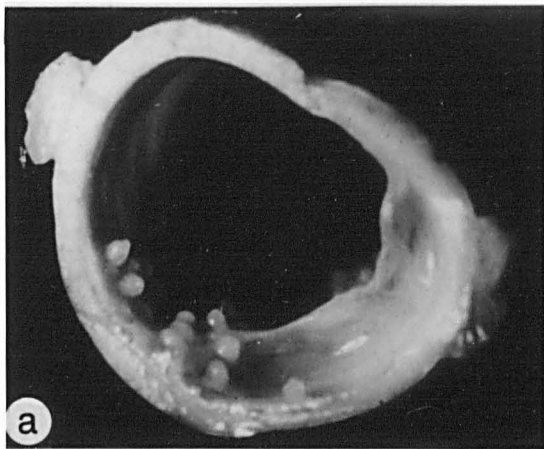


Fig 3 Gross pathological changes in the brain a of lamb affected with acute coenurosis (Fc6). The large arrowheads indicate areas where oncospheres have migrated through the cerebral cortex and in one area, the tissue has undergone liquefactive necrosis resulting in cavitation. The yellowish areas (small arrowheads) are areas of necrosis, probably arising from loss of blood supply (x 2)

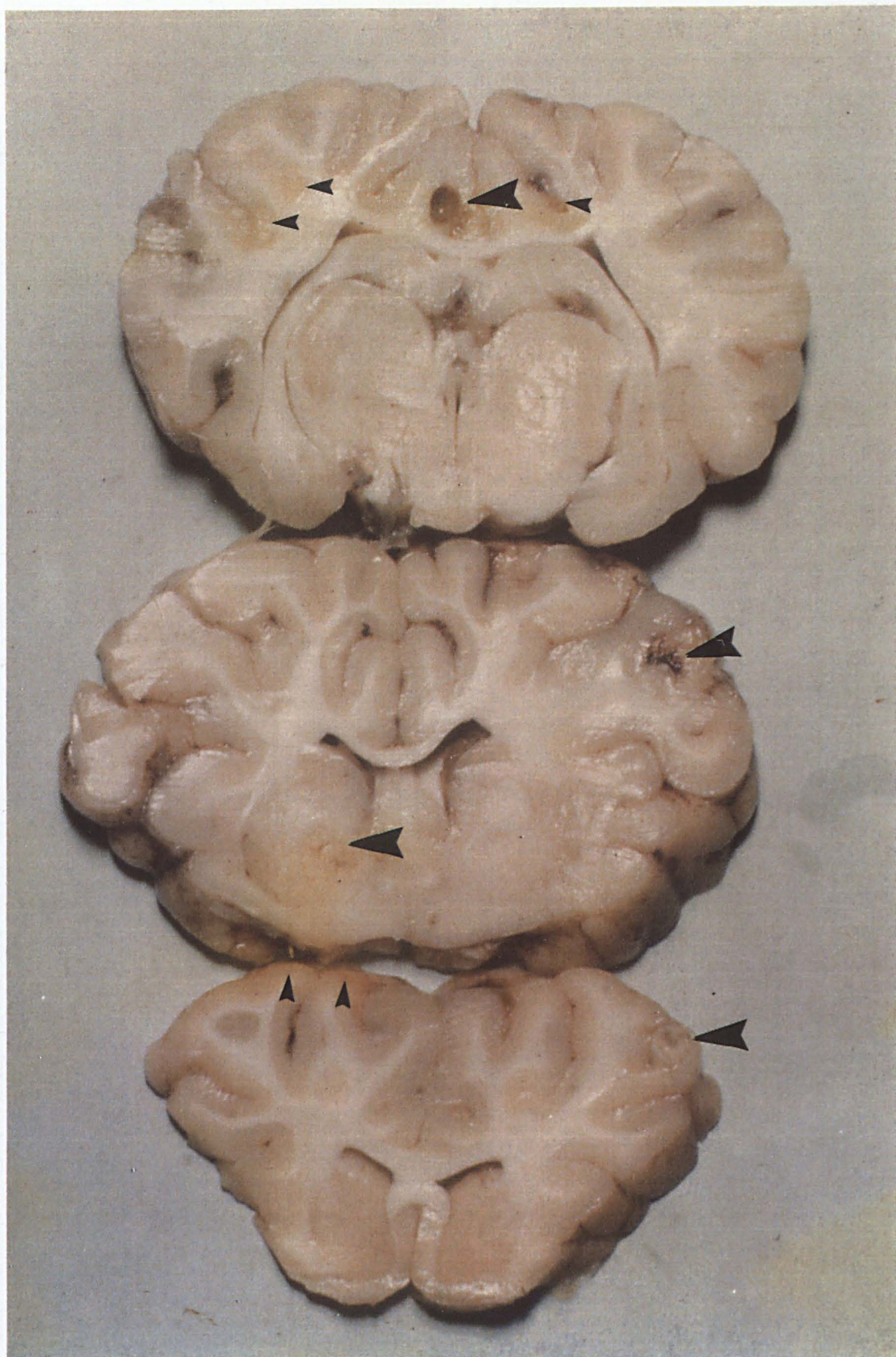


Fig 4 **Microscopic sections of visceral lesions in acute ovine coenurosis**

- a) A necrotic lesion in the mesenteric lymph node (arrowhead), with eosinophilic infiltration of the surrounding tissue (Haematoxylin [H] and Eosin [E] x 50) (x 40)
- b) A higher magnification of the necrotic centre with leucocytes and eosinophils invading the track left by the migrating oncosphere (H and E x 50) (x 100)
- c) A necrotic lesion in the kidney cortex (arrowhead) produced by a migrating oncosphere (H and E x 10) (x 40)
- d) A higher magnification of (c). The renal tubules are necrotic and the centre of the necrotic lesion is infiltrated by eosinophils (H and E x 50) (x 100)
- e) A higher magnification of the necrotic centre in (d) (H and E x 150) (x 300)
- f) A lesion in the mucosa of the duodenum showing a loss of epithelial cells (arrowhead) and infiltration of the mucosa and submucosa with eosinophils (H and E x 150) (x 100)

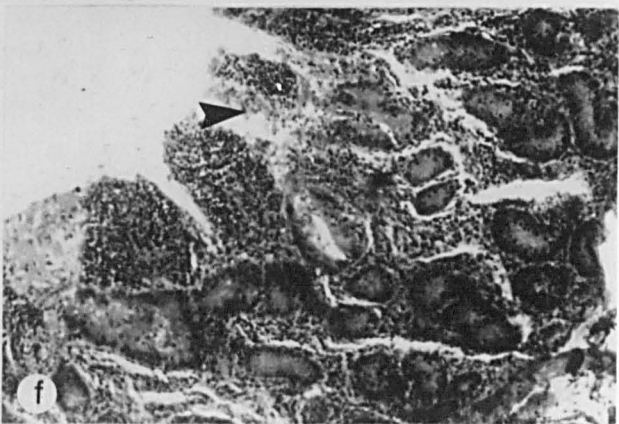
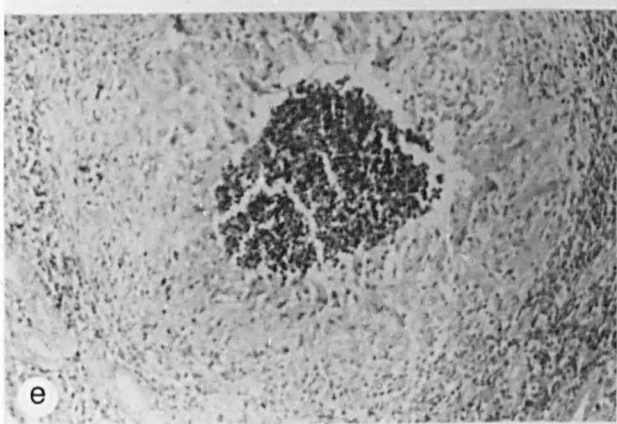
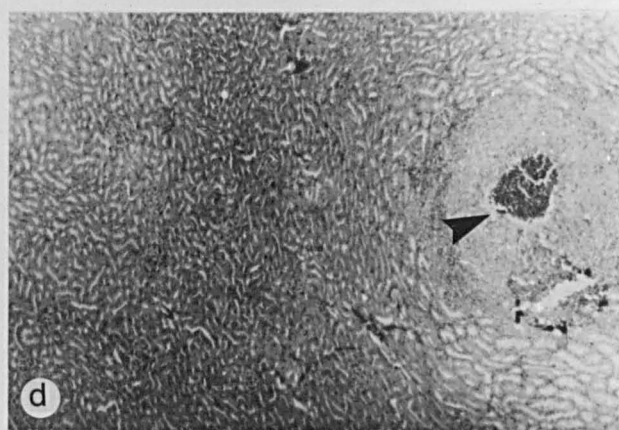
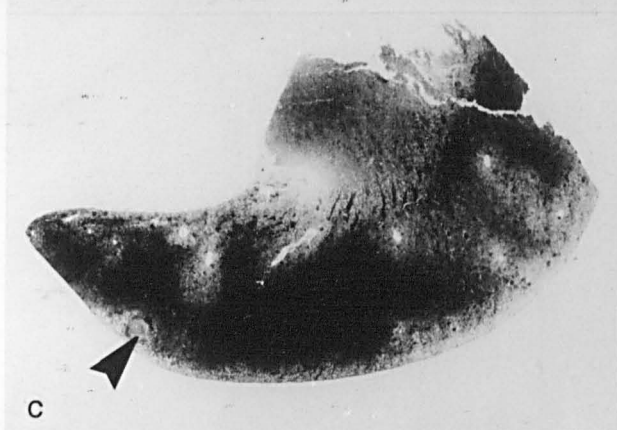
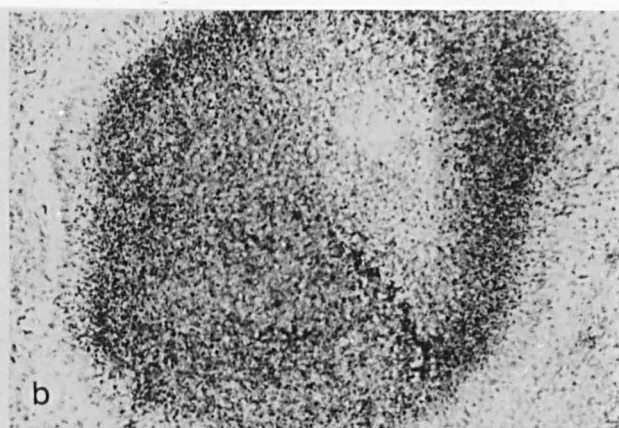
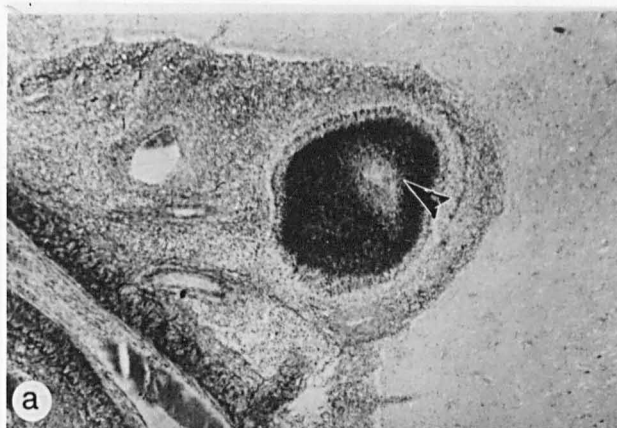


Fig 5 **Histopathological sections of lesions in the cerebral nervous system in acute ovine coenurosis**

- a) A section through the anterior cerebral hemisphere showing (arrowheads) tracks, cellular infiltration and in one site cavitation caused by oncosphere migration (Romanes x 8) (x 2)
- b) A section through a central hemisphere showing (arrowheads) cavitation and tracking following oncosphere migration (Romanes x 8) (x 2)
- c) Necrosis and eosinophilic infiltration (arrowheads) following oncosphere migration in the brain stem at the level of the corpora quadrigemina (H and E x 8) (x 4)

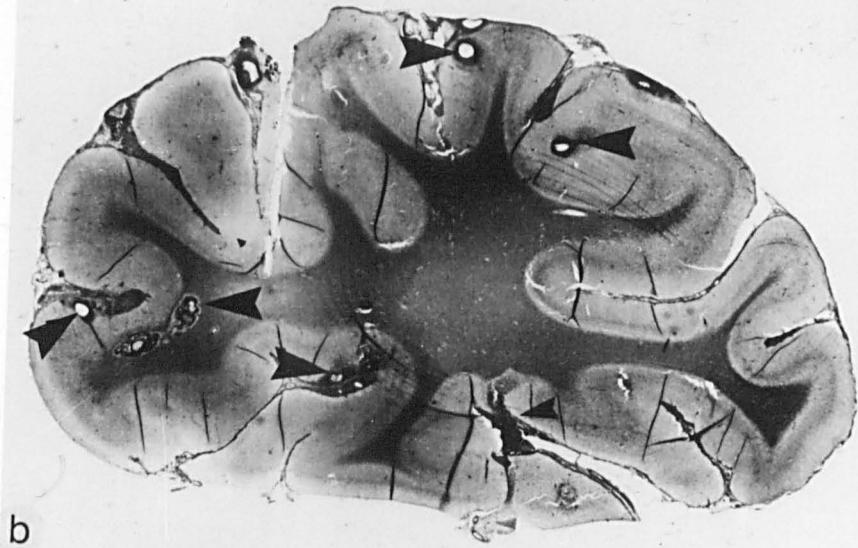
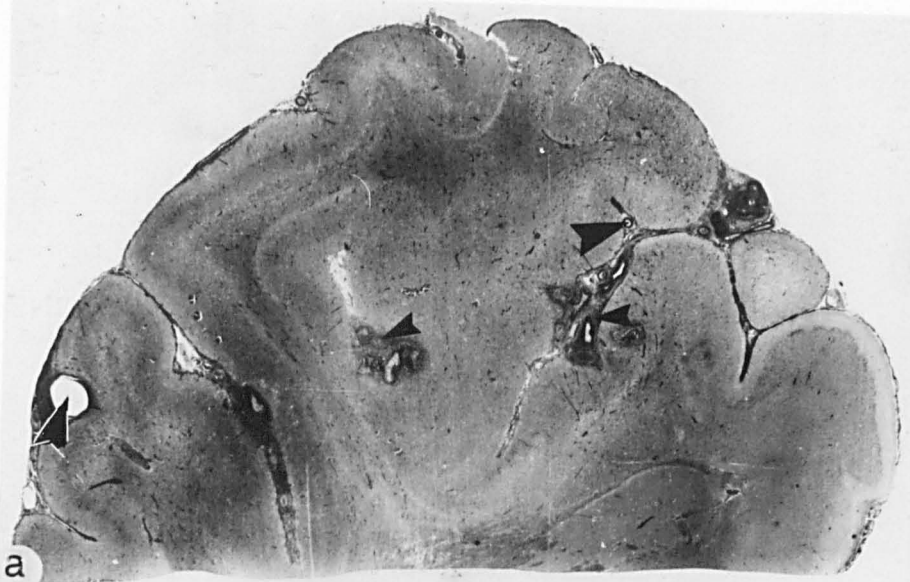
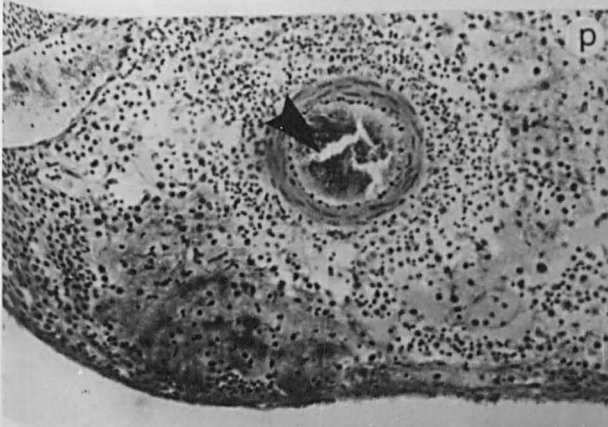
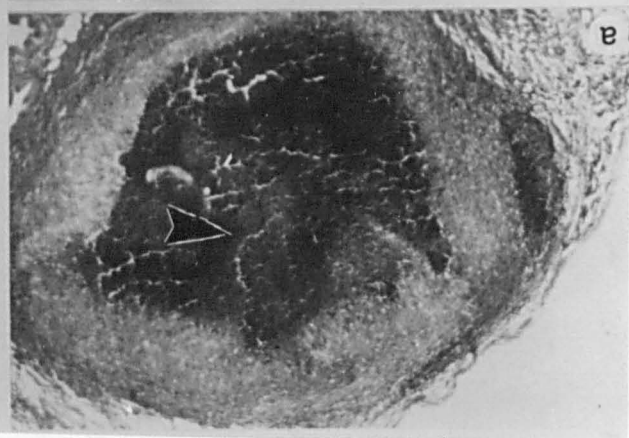
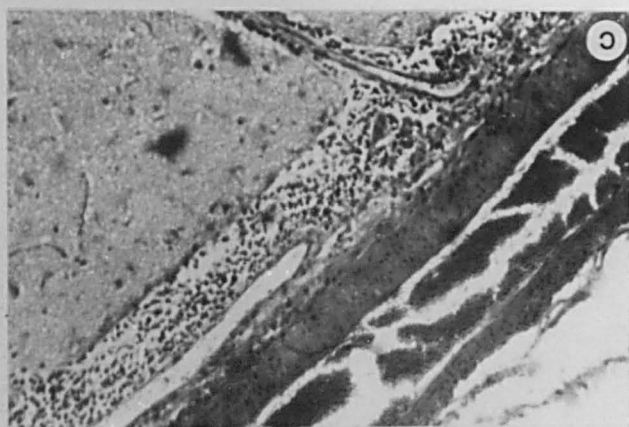
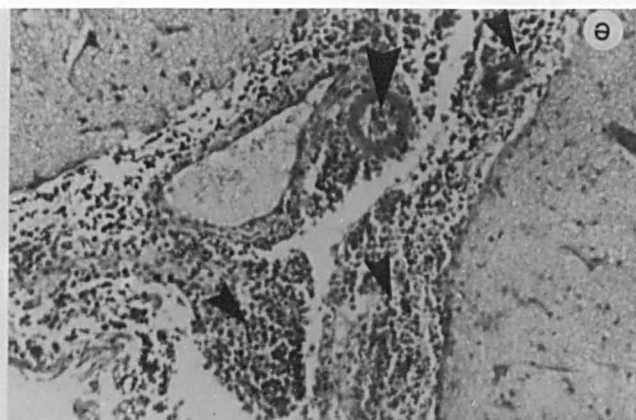


Fig 6 **Histopathological sections of lesions in the central nervous system in acute ovine coenurosis**

- a) A necrotic lesion in the cerebral cortex (arrowhead) which is infiltrated by leucocytes and eosinophils (H and E x 100) (x 50)
- b) Liquefaction of the cerebral cortex; a cavity crossed by a network of reticular fibres with some leucocytic infiltration (Romanes x 100) (x 100)
- c) Haemorrhage in the meninges with eosinophilic infiltration of the superficial cerebral cortex (H and E x 100) (x 100)
- d) Thrombus formation in one of the meningeal arterioles (arrowhead) with leucocyte infiltration of the surrounding tissue (H and E x 100) (x 100)
- e) Eosinophilic infiltration in the meninges, deep in one of the cerebral sulci (two upper arrowheads) and thrombi in two small arterioles (two lower arrowheads) (H and E x 100) (x 100)



6.3 Immunology

In 1911 Casoni demonstrated an immediate hypersensitivity reaction in the skin of patients affected with hydatid disease, following the intra-dorsal injection of hydatid cyst fluid, and since then the technique has been widely used as a diagnostic test despite certain limitations (Kagan 1968). The intra-dermal injection of Coenurus cerebralis cyst fluid into the skin of animals affected with coenurosis also produces a hypersensitive reaction and the present author has used this technique as a diagnostic test for this disease. Because of the false positive and inconclusive results obtained from time to time it was assumed that these could be attributed respectively to the presence of other cestode larvae subsequently found at post mortem examinations of animals unaffected with coenurosis on to the varying potency of cyst fluids.

Ismagilova (1958) claimed that the intradermal test was a highly reliable diagnostic test for coenurosis when antigens specially prepared from the fluid or protoscolices of Coenurus cerebralis cysts were used. He also reported that mild hypersensitivity reactions to the test were produced by hydatids and cysticerci, but these reactions could easily be differentiated from a true positive reaction to coenurosis. In view of this it was

decided to investigate Ismagilova's claims by preparing antigens according to his methods and assess their value in sheep and cattle affected with coenurosis. In addition an attempt would be made to determine the nature of the skin reaction produced by Coenurus cerebralis antigen.

A further investigation was undertaken to determine the degree of cross reaction produced in the immune responses to the larval antigens of E. granulosus, T. hydatigena and T. multiceps in laboratory animals by the complement fixation test (CFT) and to evaluate the value of the CFT in the diagnosis of coenurosis in sheep and cattle.

MATERIALS AND METHODS

T. multiceps antigen preparation

a) TMA. Fluid was harvested from a number of coenuri in the brains of sheep submitted for post mortem examination, pooled, and stored at -18°C in 0.5 ml aliquots.

b) TMB. This antigen was prepared from cyst fluid by the methods described by Ismagilova (1958). Four grams of crystalline trichloroacetic acid were added to each 100 ml of fluid collected from cysts in the brains of sheep and cattle submitted for post mortem examination. The mixture

was shaken vigorously and held at 4°C for a period of 14 hours (Ismagilova held the mixture at 4°C for 5 hours), and then centrifuged at 3000 rpm for 15 minutes to deposit the precipitate. The supernatant fluid was decanted, its pH adjusted to 7.0 with sodium hydroxide solution and dialysed overnight against tapwater. After dialysis, three times the volume of 95% methyl alcohol was added and the precipitate removed by centrifugation at 3000 rpm for 15 minutes. The precipitate was dissolved in distilled water and three times the volume of 95% methyl alcohol added and the precipitate removed once more by centrifugation at 3000 rpm for 15 minutes, which was then dried in an incubator at 37°C for 24 hours. The dried powder, the allergen, was dissolved in normal saline at the rate of 0.2 gm of dried precipitate to 150 ml of normal saline a few drops of merthiolate added as a preservative and stored at 4°C.

c TMC. The third antigen was prepared from the protoscolices removed from coenuri recovered from the brains of sheep and cattle submitted for post mortem examination, by the method described by Ismagilova. Protoscolices were removed from the coenuri by scraping the inside wall with a scalpel and dried in an incubator overnight at 37°C. One gram of the dried protoscolices was ground up in a Griffiths tube with 30 ml of normal saline, the pH of the liquid adjusted to 4.5 with

hydrochloric acid, 0.1% w/v pepsin (BPC) was added and incubated at 37°C for 24 hrs. The pH was adjusted to 3.5 with hydrochloric acid, three times the volume of 95% methyl alcohol added and the mixture centrifuged at 3000 rpm for 15 minutes. The deposit was resuspended in distilled water and precipitated once more by the addition of 95% methyl alcohol and centrifuged at 3000 rpm for 15 minutes. The precipitate was dried for 24 hours at 37°C and 0.2 gm of the precipitate dissolved in 150 ml of normal saline, a few drops of merthiolate was added as a preservative and stored at 4°C.

T. hydatigena antigen preparation

This antigen consisted of fluid collected from Cysticercus tenuicollis cysts removed from sheep at slaughter, pooled and stored at -18°C.

E. granulosus antigen preparation

Fluid from fertile hydatid cysts in the lungs and livers of sheep slaughtered at a local abattoir was pooled, centrifuged at 3000 rpm for 15 minutes to remove the hydatid "sand" and stored at -18°C.

Preparation of antisera

Antisera were raised in rabbits against each of the antigens prepared.

New Zealand White rabbits weighing approximately 30 kilos received 0.1 ml of the antigens on the first day by intravenous injection and then every other day the dose was increased so that by the fourteenth day each rabbit received 1.0 ml of the antigen. Each rabbit was test bled at 21 and 28 days after the first injection had been administered and the sera subjected to the CFT for an assessment of the immune response, which in each proved satisfactory. A further 1.0 ml of antigen was administered subcutaneously 28 days after the first injection and 14 days later the rabbits were anaesthetised with chloroform and bled out. The sera were separated by centrifugation, divided into 1 and 2 ml aliquots and stored at -18°C .

Complement

Freeze dried guinea pig serum (Wellcome Reagents Ltd) was used as the source of complement in all the CFTs and was reconstituted according to the manufacturer's instructions.

Haemolytic serum

Horse antish sheep haemolytic antiserum preserved with 30% glycerol was obtained from a commercial source (Wellcome Reagents Ltd).

Sheep red blood cells

Sheep red blood cells in Alsevers solution were obtained either from Wellcome Reagents Ltd or from Difco Laboratories Ltd, and stored at 4°C. On the day of use, the cells were washed with CFT buffer (Oxoid Ltd), 5 ml of red cells and 5 ml of buffer were mixed together and centrifuged at 2000 rpm for 5 minutes and the supernatant discarded. The packed cells were resuspended in buffer and centrifuged once more and if necessary was repeated until the supernatant was colourless. The cells were then resuspended in buffer to give a 3% suspension ready for use in the CFT.

CFT buffer (Oxoid Ltd)

The constituents of the buffer were

| | | | |
|--------------------|----------|--------------------|----------|
| Sodium chloride | 85 gms | Barbituric acid | 5.75 gms |
| Sodium barbiturate | 3.75 gms | Magnesium sulphate | 2.0 gms |
| Calcium chloride | 0.29 gms | | |
| Distilled water | 2 litres | | |

The buffer was stored at 4°C in a glass container and diluted 1 in 5 in distilled water immediately before use.

CFT

The test was a standard CFT used at MAFF laboratories and is described in the Manual of Veterinary Investigation Laboratory Techniques (Reference Book 363 and in Appendix 10.3). It was carried out in 100 well MRC plastic trays and before each test was set up, the optimum concentrations of haemolytic serum, complement and each antigen were determined. The test sera were inactivated at 60°C for 30 minutes in a water bath and tested in doubling dilutions from 1/4 to 1/2048 in 0.2 volumes.

Skin hypersensitivity tests

The antigens were injected into the skins of the caudal fold of sheep and cattle. The thickness of the fold was measured with a caliper calibrated in millimetres immediately before injection and three hours later.

RESULTS

(A) Skin hypersensitivity reactions

Two antigens only were used because there appeared to be no significant difference between TMA and TMB in a preliminary exercise. The antigens, TMA and TMC were injected alternately into the caudal fold of suspected coenurosis cases and animals which were apparently normal.

The sheep experimentally infected and referred to in earlier chapters, TM3, TM4, TM5 and TM6, were teated on the day euthanasia was carried out, ie between 11 and 19 days post infection and none reacted to the antigens. TM1 was tested with TMA at 21, 60 and 90 days post infection and on all occasions an increase in thicknes of 3 mm was recorded (Fig 1a). TM2 was tested at the same intervals and at 60 and 90 days post infection there was an increase of 15 mm in skin thickness, with an obvious reddening of the injection site (Fig 1b).

The results of hypersensitivity tests on confirmed field cases of coenurosis in sheep and cattle and on normal in contact animals are set out in Tables 1 and 2.

All sheep suspected of being affected with coenurosis tested with TMA had skin thickness increases of 3 - 5 mm. Of the 15 normal sheep tested with this antigen 10 had skin thickness increases of less than 3 mm, three animals had increases of 3 - 5 mm and another two sheep, one of which subsequently developed coenurosis had skin thickness increases of more than 5 mm. All 7 cattle affected with coenurosis had increases of more than 3 mm and six of these increases of mor than 5 mm. Three of the 10 normal cattle had increases of less than 3 mm and of the remainder two had increases of more than 5 mm.

Of the 25 sheep with clinical coenurosis tested with TMC, 24 had increases of more than 3 mm and one of these an increase of 11 mm, but 8/15 of the normal sheep had increases of more than 3 mm. All 5 cattle affected with coenurosis had skin increases in excess of 5 mm and one of these animals, a 12 month old Hereford bull developed a 22 mm increase within 10 minutes of injection (Fig 1c). Of the normal cattle tested, 5 had increases in excess of 5 mm and the remainder between 3 and 5 mm.

(B) The nature of the hypersensitivity reaction

Although high levels of reaginic (homocytotropic) antibody had been demonstrated in individual animals infected by helminths in 1974, the only homocytotropic antibody to a cestode antigen that had been demonstrated in sheep was to E. granulosus. As a result an investigation to determine whether a homocytotropic antibody to T. multiceps antigen developed in sheep was undertaken and this was successfully demonstrated and reported (Williams 1975).

(C) Serology

(i) CFT on rabbit sera

Prior to immunisation all rabbit sera were negative to all the antigens at a dilution of 1/4. The CFT titres obtained in tests with homologous and heterologous antigens are set out in Table 3.

Antigens TMA and TMB produced higher titres than TMC in rabbits. When TMA and TMB antigens were tested against TMB and TMA antisera respectively the titres were the same. TMC antigen produced much lower titres against TMA and TMB antisera, as well as against its homologous serum. When all the antigens were tested against all the antisera, it was evident that there was a high degree of cross reaction between the three cestode species.

(ii) CFT on sheep sera

a) Experimental sheep

The sera of all six sheep (TM1 - 6) were tested against TMA and before experimental infection the titres were less than 1/32. The sera from the animals (TM3 - 6) that developed acute coenurosis were tested between 11 and 16 days after infection, two of which had titres of 1/64, one 1/28 and the fourth 1/256. TM1 did not develop clinical coenurosis, but 60 days post infection the titre was 1/256

and at 150 days was at same level. The CFT titre of the serum from TM2 was 1/256 at 30 days post infection and at 140 days post infection it had risen to 1/512.

The sera from these animals were also tested against E. granulosus and T. hydatigena antigens. In all cases the E. granulosus titres were the same as for TMA antigens, but the titres against T. hydatigena antigen were one or two dilutions lower.

b) Field cases of coenurosis

The sera from 21 sheep, 3 affected with acute and 18 with chronic coenurosis were subjected to the CFT against the antigens of T. multiceps (TMA), E. granulosus and T. hydatigena and the results are set out in Table 4.

Sera from animals with chronic disease had titres of 1/128 - 1/256 against the T. multiceps antigen, titres of 1/64 - 1/256 against T. hydatigena antigen and titres of 1/64 - 1/128 against E. granulosus antigen. Sera from the acute cases were very much lower namely 1/16 - 1/32 against T. multiceps, 1/8 - 1/16 against T. hydatigena and 1/8 - 1/32 against E. granulosus. Sera obtained from 15 normal animals on farms where coenurosis had recently been

confirmed, had titres of 1/128 - 1/512 against T. multiceps, 1/128 - 1/256 against T. hydatigena and 1/128 - 1/512 against E. granulosus (Table 4).

DISCUSSION

Gemell and Macnamara (1972), Rickard and Williams (1982) and Rickard (1983) in reviewing the literature on the immune response to metacestodes demonstrated the complexity of the subject and referred to the fact that certain species of cestodes have antigens in common. The main objective of this part of the study was to determine whether a reliable diagnostic test based on the immunological response to T. multiceps antigens could be developed for use by veterinarians engaged in farm animal practice.

Ismagilova (1958) following a prolonged period of experimental work advocated the use of the intradermal allergic test for the diagnosis of coenurosis, claiming that by using purified antigens a specific diagnosis could be made. His method of preparation of antigens was closely followed at Carmarthen and disappointingly the two antigens prepared by his method performed no better than untreated Coenurus cerebralis fluid in subsequent tests.

Ismagilova (1958) performed the test on the thick skin of the upper eyelid, a procedure which is not easy to carry out on sheep and cattle and requires complete restraint of the animals. To avoid or overcome such problems of restraint and to reduce the stress on animals, the allergen was injected into the skin of the caudal fold. Such a site has certain advantages, readily accessible, requires no clipping of hair or cleaning, skin thickness is easily measured and animals seldom resent any interference at this site including the injection of allergen. The injection however must be carried out carefully, as the skin is very thin, to avoid introducing the allergen into the subcutaneous tissues.

The increases in skin thickness reported by Ismagilova (1958) were not matched by those recorded in the present study, despite the fact that the antigens were produced in exactly the same manner. It may well have been that the differences in skin sensitivity of the eyelid and caudal fold were partly responsible, but this is unlikely because it has been demonstrated that skin sensitivity to tuberculin in the diagnosis of tuberculosis is not significantly different in the skin of the caudal fold and the skin at other sites. Ismagilova considered that an increase in skin thickness of less than two centimetres was negative for coenurosis, 2.1 - 2.5 cms was doubtful and increases of 2.6 cms or more positive. In the present

study the maximum response in sheep affected with coenurosis was 11 mms, with either of the two antigens. Only one affected sheep had an increase of less than 3 mm with either of the antigens. Thus if one accepted that a positive response to T. multiceps antigen was an increase of 3 mm or more, then four of the 15 normal sheep tested with TMA were positive and 8/15 normal sheep tested with TMC. All 5 clinical cases of coenurosis cases in cattle gave increases of more than 5 mm with TMC, but so did 5/10 normal cattle. Similarly all 7 cattle affected with coenurosis tested with TMA had increases of more than 3 mm and 7/10 normal cattle had similar increases.

From these data an estimate of the sensitivity and specificity of the two antigens could be made, but these two terms must be defined in the first instance. The sensitivity of an antigen may be defined as its ability in the allergic test to correctly identify animals with coenurosis. The percentage sensitivity was calculated as follows

$$\frac{\text{True positive results}}{\text{True positive results} + \text{false negatives}} \times 100$$

The specificity of an antigen may be defined as its ability to correctly identify those animals which are not affected with coenurosis and the percentage specificity calculated as follows

True negative results

x 100

True negative results + false positives

On this basis the percentage sensitivity and specificity of TMA in sheep were 100 and 71.4 respectively. In cattle the comparable figures were 100 and 30. For antigen TMC percentage sensitivity and specificity were 96.1 and 46.7 in sheep whilst in cattle the values were 100 and 0. The sensitivity of both antigens in cattle and sheep were thus high, but in view of the low specificity the allergic test as a diagnostic tool was of limited value.

Dyson and Linklater (1979) also considered that the allergic skin test was of limited value in the diagnosis of coenurosis. In addition, Skerritt and Stallbaumer (1984) also reported that the intradermal allergic test did not contribute usefully to the diagnosis of coenurosis. Both groups of authors used coenurus fluid as an allergen, which was injected into the skin of the neck and a visual assessment was made of the reaction some hours later.

The reasons for the low specificity of the test have not been fully clarified. Ismagilova (1958) reported that sheep and cattle infected with other metacestodes reacted to T. multiceps antigen. Schantz (1973) and Williams (1975) demonstrated reaginic antibodies to E. granulosus

and T. multiceps respectively and since then reaginic antibodies have been demonstrated in a variety of animals infected with a number of cestode species. It is significant that Schantz (1973) demonstrated reaginic antibody to E. granulosus in sheep infected with either larvae of E. granulosus or T. hydatigena. Thus it would appear that these two cestode species have antigens in common capable of stimulating the production of this antibody. In view of cross reactions demonstrated in the CFT between T. multiceps, T. hydatigena and E. granulosus it was likely that the results of the allergic tests with T. multiceps may have been influenced by the presence of larvae of the other two cestodes in the cattle and sheep tested. In addition to this it was also possible that the normal animals tested in the herds and flocks had been infected with the larvae of T. multiceps and these had subsequently failed to develop.

It was evident from the results obtained in the CFT's on rabbit sera and sera from cattle and sheep that the test was incapable of correctly identifying all animals that were not affected with coenurosis. Dyson and Linklater (1979) concluded that the haemagglutination test for T. multiceps was of little value in diagnosis and similarly Hackett, Willis, Herbert and Edwards (1981) found that the enzyme-linked immunosorbent assay (ELISA) test and the indirect haemagglutination (IHA) test did not distinguish

between lambs infected with T. hydatigena metacestodes and those which were not when tested with antigens prepared from either T. hydatigena of T. multiceps. These authors concluded that until an antigen is found which can distinguish between the various taeniid species and clearly delineate infected from uninfected animals, the ELISA, IHA and other serological tests are of limited value, a point of view with which I am in full agreement.

| No of animals tested | Disease category | Number of animals with increases in thickness of caudal fold of | | | Range mm |
|----------------------|------------------|---|----------|--------|----------|
| | | < 3 mm | 3 - 5 mm | > 5 mm | |
| 26 sheep | Coenurosis | 0 | 26 | 0 | 3 - 5 |
| 15 sheep | Normal | 10 | 3 | 2* | 1 - 8 |
| 7 cattle | Coenurosis | 0 | 1 | 6 | 4 - 10 |
| 10 cattle | Normal | 3 | 5 | 2 | 2 - 12 |

* one sheep subsequently developed coenurosis

Table 1 Results of intradermal tests in sheep and cattle with T. multiceps antigen TMA

| No of animals tested | Disease category | Number of animals with increases in thickness of caudal fold of | | | Range mm |
|----------------------|------------------|---|----------|--------|----------|
| | | < 3 mm | 3 - 5 mm | > 5 mm | |
| 26 sheep | Coenurosis | 1 | 12 | 13 | 2 - 11 |
| 15 sheep | Normal | 7 | 6 | 2 | 2 - 6 |
| 5 cattle | Coenurosis | 0 | 0 | 5 | 7 - 22 |
| 10 cattle | Normal | 0 | 5 | 5 | 3 - 8 |

Table 2 Results of intradermal tests in sheep and cattle with T. multiceps antigen TMC

| Antiserum | Antigen | | | <u>T. hydatigena</u> | <u>E. granulosus</u> |
|----------------------|---------------------|--------|-------|----------------------|----------------------|
| | <u>T. multiceps</u> | | | | |
| | TMA | TMB | TMC | | |
| <u>T. mult.</u> TMA | 1/1024 | 1/1024 | 1/256 | 1/512 | 1/1024 |
| TMB | 1/1024 | 1/1024 | 1/256 | 1/256 | 1/1024 |
| TMC | 1/256 | 1/256 | 1/512 | 1/128 | 1/256 |
| <u>T. hydatigena</u> | 1/512 | 1/256 | 1/128 | 1/512 | 1/256 |
| <u>E. granulosus</u> | 1/1024 | 1/1024 | 1/256 | 1/512 | 1/1024 |

Table 3 CFT titres in tests of homologous and heterologous antigens against antisera prepared against T. multiceps, T. hydatigena and E. granulosus

| Class of sheep | No of sheep | Antigen | | |
|---|-------------|------------------|----------------|-----------------|
| | | <u>T. mult.*</u> | <u>T. hyd.</u> | <u>E. gran.</u> |
| Chronic coenurosis | 18 | 1/123-1/256 | 1/64-1/256 | 1/64-1/128 |
| Acute coenurosis | 3 | 1/16 - 1/32 | 1/8 - 1/16 | 1/8 - 1/32 |
| "Normal" (not affected with coenurosis) | 15 | 1/128-1/512 | 1/128-1/256 | 1/128-1/512 |

* T. multiceps - TMA antigen

Table 4 CFT titres with T. multiceps, T. hydatigena, E. granulosus antigens in 21 sheep affected with coenurosis and in 15 "normal" sheep

Fig 1 Hypersensitivity skin reactions in caudal fold (arrowheads) following the intradermal injection of Taenia multiceps antigen

- a) Lamb TM1. Increase in skin thickness of 3 mm after injection with antigen TMA (see text) (x 1)
- b) Lamb TM2. Increase in skin thickness of 15 mm after injection with antigen TMA (x 1)
- c) Hereford Bull. Increase in skin of 22 mm after injection with antigen TMC (x 1)



7 GENERAL DISCUSSION

The main aims and objectives of the studies as set out in 1.1, were to determine the prevalence of coenurosis in sheep flocks in Dyfed and to identify as far as possible the principal epizootiological factors responsible for its prevalence and indeed persistence in individual flocks. The results obtained in each part of the investigations provided valuable information, which clearly identified the major factors which allowed the transmission of infection from the carnivorous definitive hosts to sheep and indicated how the disease could be eradicated from individual flocks. Some of these results are further discussed and attention is drawn to the needs for additional investigations.

Examination of adult and larval specimens of Taenia multiceps confirmed that it was a valid species on morphological grounds, and in most respects the morphology was similar to that described by other authors. However the muscular pad on the anterior wall of the vagina, which Verster (1969) considers to be a distinguishing feature in identifying the species from Taenia serialis was not present in the adult cestodes examined at Carmarthen. In view of this, further strains of the cestode recovered from dogs and hounds in Great Britain should be examined to determine whether, as in African strains examined by Dr Arlene Jones, the pad may or may not be present even in

segments from the same strobila. Furthermore experimental studies on known specimens of T. multiceps and T. serialis would resolve the conflicting views expressed by Esch (1964, 1967), Esch and Self (1965) who consider that on morphological grounds and limited experimental work, that the two cestodes are different strains of the same species capable of infecting a number of intermediate host-species, and Verster (1969), who maintains that on morphological grounds alone that they are distinct species. In this context studies on protein and enzyme profiles and DNA sequences as described by McManus and Smyth (1975) and McManus and Simpson (1985) for Echinococcus granulosus subspecies would be helpful.

Despite its limitations, the postal survey of flocks in Dyfed clearly established that coenurosis was prevalent in the vast majority of flocks and that it was an important cause of morbidity and mortality in many, thereby confirming the views previously expressed by farmers and practising veterinary surgeons. There was no reason to believe that the situation in other flocks in Dyfed was different.

Although the average annual flock loss was only 2.5 - 3.0 per cent, which in comparison with losses caused by some other diseases was unremarkable, the economic losses to the sheep industry should not be ignored. Because it was

not possible to gain access to individual farm accounts the financial losses in individual flocks should not be easily calculated, although three farmers volunteered information, which is referred to later in this chapter.

It was apparent that animals under 12 months old were more frequently affected by coenurosis and as all male animals other than the small number required for breeding purposes, were sold either fat or for further fattening between four and nine months old, it was assumed that 75% of those affected were female. On this basis it was calculated that 604 - 724 male lambs were lost, which at 1976 prices represented a loss of £10,872 - £13,032 and 1811 - 2174 female lambs at 1976 prices valued at £39,842 - £47,828, a total direct loss of £50,714 - £60,860 or £92 - £110 for each of the 552 flocks in the survey and £135 - £162 for each of the 375 infected flocks. However such a calculation did not take into account the indirect losses arising out of deaths or slaughter of animals, including disruption of breeding programmes, purchase of replacement stock, delays in drafting older breeding stock and loss of subsidies. Thus the financial losses arising from deaths due to coenurosis were better illustrated by information provided by individual farmers.

One flock owner, a well known producer of Welsh half breds, in much demand at the time, had lost more than 120 females, lambs and yearlings, over a six year period, which severely disrupted his Welsh half bred breeding programme. He estimated that his losses over this period had amounted to no less than £4,500, mainly due to loss of sales and purchase of pedigree replacement stock. Another who participated in the regular survey of farm incomes carried out by the Agricultural Economics Department, University College of Wales, Aberystwyth reported that his flock losses from coenurosis were consistently between 3 and 5 per cent/year and that it had been calculated by the Department that the direct and indirect economic losses amounted to an average of £240 - £300 per annum. A third farmer, whose flock was participating in the Meat and Livestock Commission Flock Recording Scheme considered that his losses from coenurosis were minimal and he believed that his total economic loss over a period of three years did not amount to more than £200 and half this sum was attributable to the purchase of a replacement pedigree Suffolk ram lamb.

Although there was no doubt about the economic importance of coenurosis, no attempt was made, indeed could not be made, to obtain more accurate assessments of the economic consequences of coenurosis outbreaks. The examples quoted above indicate that a more critical study is warranted.

The importance of coenurosis in purchased ewe lambs and yearling ewes was stressed by a number of farmers and others reported on the occurrence of coenurosis in sheep overwintered on dairy farms when they returned to the home farm. Such reports had previously been treated with a certain degree of scepticism, but the flock owners presented irrefutable evidence that could not be ignored. Therefore the control of coenurosis even in flocks which do not purchase breeding stock other than rams, is not entirely within the flock owners province, and the need for dogs on wintering farms to be regularly treated with taeniicides and prevented from consuming sheep heads cannot be overemphasised.

Two significant findings from the survey on feeding and management of farm dogs include the high proportion of dogs that consumed uncooked meat and offal and the equally high proportion that were never or only occasionally treated with taeniicides. Allowing dogs to consume raw meat and offal was in the main attributable to a failure by farmers and shepherds to understand the implication of such actions and the life cycles of canine cestodes. Although a significant number of flock owners insisted that their dogs did not consume uncooked meat and offal, treatment with Cestarsol produced evidence to the contrary. Even in the face of such evidence some individuals vehemently denied feeding such items or

allowing dogs to scavenge on carcasses. The confinement of dogs when not required for shepherding would have prevented much of the scavenging.

As the study was specifically designed to elucidate certain aspects of the epizootiology of coenurosis, information on how dogs acquired T. multiceps infection was particularly important. Over a period of many years the author has observed the scavenging of sheep carcasses on sheep walks and common grazings by birds, dogs and on occasions, foxes. The abdominal and thoracic cavities and organs are the first sites to be scavenged, although birds only do so after removal of the eyes and anterior portion of the tongue. Thereafter the skeletal muscles are attacked, but the head is seldom removed from the carcass and invariably the cranium is left intact. My perception therefore was, and still is, that whilst E. granulosus, T. ovis and T. hydatigena infection in dogs and foxes could be acquired through scavenging, T. multiceps was not, and that the deliberate feeding of sheep heads was more likely to be the main, if not sole factor responsible. Confirmation of this was obtained from the farmers on both sheep rearing and wintering farms. The fact that many heads of fat lambs slaughtered for human consumption contained recognisable coenuri of T. multiceps and that none showed nervous signs illustrated the danger of

feeding heads from such animals. Furthermore some farmers admitted to the deliberate feeding of heads from sheep known to be affected with coenurosis.

The disappointingly low proportion of dogs that were treated with taeniocides on a regular basis was equally significant. The two main reasons put forward for not treating was the cost and the unawareness of the need for regular treatment. Whilst it was difficult to accept that the cost of treatment was in any way prohibitive, farmers insisted that under the prevailing economic pressures which faced the sheep industry, the cost of treatment had to be given due consideration. Some admitted that when Cestarsol was available dogs were treated at a cost of about 50 pence, per dog per annum, but after its withdrawal treatment with bunamidine would cost at least £4, per dog per annum, which was equivalent to the profit from one ewe. Even when the cost benefit of treatment was explained, there was a great deal of reluctance to accept such evidence, although it must be admitted that some owners did so. It must be emphasised that when economic circumstances are unfavourable as they were in the mid 1970s, animal health programmes, such as vaccination and anthelmintic treatment of farm livestock are given a low priority and the treatment of dogs even a lower priority.

O'Sullivan (1981) draws attention to the reluctance of farmers in Powys to treat dogs regularly because of what they consider to be the high cost of praziquantel.

Carcase disposal posed a difficult problem for most sheep farmers, especially those on upland farms where soil depth precluded burial of carcasses, and those on farms remote from knackeries and hunting kennels. The inability of knackeries to provide and maintain a regular sheep carcase collection service on economic grounds, was overcome by the acceptance of sheep carcasses by hunting kennels. Indeed hunting kennels were the only means of carcase disposal available to some farmers. With the recognition of Bovine Spongiform Encephalopathy (Wells, Scott, Johnson, Gunning, Hancock, Jeffrey, Dawson and Bradley 1987) and its association with the feeding of compound feeding stuffs containing sheep derived protein (Wilesmith, Wells, Cranwell and Ryan 1988, Wilesmith, Ryan and Atkinson 1991), the inclusion of meat and bone meal derived from ruminants in feeding stuffs for feeding to ruminants is now prohibited. As a result knackeries have suffered a severe economic crisis. Renderers who purchased meat, bones and offal from knackeries for producing tallow and meat and bone meal are now unable to dispose of meat and bone meal in vast quantities to animal feed compounders, their only outlet being those compounders who produce feeding stuffs for the poultry and

pig industries. Knackeries in the mid 1970's paid £3 per bovine carcase or £10 - £15 for live cattle and horses on collection from farms, but now because of the reduced demand from renderers, they levy a charge for horse and cattle carcase collection in Dyfed which varies from £25 - £50 depending on the distance travelled. A charge of up to £5 per sheep may be charged when carcases are collected by knackers during a visit to neighbouring farms to collect large animal carcases. Consequently hunting kennels are now under severe pressure to accept a much higher volume of carcases, including a much higher proportion of cattle and horse carcases. Sheep farmers are therefore likely to find it more difficult to dispose of carcases in future and the feeding of uncooked meat and offal to and scavenging by dogs may become more common than hitherto.

In view of the frequency of feeding of uncooked meat and offal by farm dogs and the low proportion of farm dogs regularly treated with anthelmintics, it was not surprising to find a high prevalence of cestode infection. The presence of T. multiceps in dogs on sheep rearing farms on which coenurosis was recorded was highly significant. The finding of this cestode in dogs on wintering farms was initially thought to be of little significance, but in view of the fact that infection could persist for six months or longer and that older sheep can

become infected, there was no doubt that transmission to the next batch of overwintered sheep could and did occur in some instances.

The levels of cestode infection in the ten infected packs of hounds examined were unexpectedly high and as in farm dogs was closely linked to feeding and anthelmintic treatment. Andrews and Lancaster (1990) draw attention to the potential public health risk posed by hounds infected with E. granulosus equinus although they acknowledge that there is no direct evidence to link infection in hounds and man. These authors appear to have overlooked the wider implications of E. granulosus and other cestode infections, namely the dissemination of infection and the animal health as well as the public health problems arising from such dissemination. The same authors put forward suggestions to eliminate infection in hounds, namely the feeding of cooked meat and offal only and regular anthelmintic treatment. Such advice has on a regular basis been given by MAFF veterinary officers to the Masters of Foxhounds Association and individual MFH's received such advice from the association. However "farmer" packs are not members of the Association but advice on feeding and anthelmintic treatment was provided by the author to the kennelmen of the packs studied. Although the kennel men readily accepted the advice on anthelmintic treatment they were reluctant to make any

change to the feeding practice of hounds. The purchase, installation and maintenance of cooking equipment was considered to be too costly and the only concession they were prepared to make was to trim offals which were obviously infected with metacestodes and to discard heads. The feeding of canned or dried meat was considered to be impractical on grounds of cost.

The prevalence of cestodes indicated that pet dogs and foxes were not important sources of infection for farm livestock. Unsurprisingly, the burdens of cestodes with intermediate stages in rodents and lagomorphs were generally higher in foxes than in hounds or farm dogs.

Acute and chronic coenurosis were reproduced in experimentally infected lambs and the acute disease appeared to be related to the number of oncospheres which reached the brain. Most outbreaks of acute disease occur in unweaned lambs and it is possible that a functioning reticular groove allows oncospheres to enter the abomasum duct rather than the rumen. Consequently more oncospheres reach the duodenum than would otherwise do so if they entered the rumen. Although the lambs in the present study had been weaned, the reticular groove was likely to be still functional and allowed the suspension of oncospheres to enter the rumen directly.

Investigations into the immunological aspects of the disease were conducted with the aim of developing a diagnostic test which could be adopted for routine use at the laboratory and by practising veterinary surgeons. Despite the preparation of more refined antigens for use in the allergic and complement fixation test, neither are of any real value. Skerrit and Stallbaumer (1984) reported that the interpretation of clinical signs was the best method of diagnosis. Doherty, McAllister and Healy (1989) used ultrasound for locating a coenurus in the brain of a lamb, but this procedure is not readily available to practising veterinary surgeons. Diagnosis of the acute forms can as demonstrated in chapter 6.1 be readily confirmed by the examination of cerebro-spinal fluid which shows an increased cell counts, a high proportion of these being eosinophils.

The treatment of chronic coenurosis has and continues to be based on surgical removal of the coenurus and Skerrit and Stallbaumer (1984) claim a success rate of between 54 and 81 per cent. Verster and Tutin (1982a) and Eslami and Bazargani (1986) report on the successful treatment of animals with clinical signs of chronic coenurosis with praziquantel at a dose rate of 100 mg/kg body weight for 2 - 5 days. This compound is only effective against coenuri of 12 weeks or more development. A trial of this preparation which is not licensed for use in sheep would

be useful and if shown to be effective could be used as an alternative to surgical removal, which due to the location of the coenurus, is not always possible, and would in any case avoid damaging the brain which is inevitable during surgery.

A discussion of ovine coenurosis would not be complete if no reference was made to immunisation of sheep against infection with T. multiceps. Verster and Tutin (1982b, 1987) and Edwards and Herbert (1982a) demonstrated that antigens derived from the oncospheres of T. multiceps were capable of stimulating protection in lambs and suggested that a vaccine developed from such antigens could in conjunction with routine anthelmintic treatment of dogs be useful in control and possible eradication. The development and subsequent marketing of such a vaccine in Great Britain would only be allowed under the supervision of the Animal Medicines Directorate of MAFF and any commercial organisation would have to demonstrate that the vaccine was up to certain standards of efficacy, safety and purity. No commercial organisation would undertake the development of a vaccine unless there was clear demand and it was assured of sufficient sales to cover development costs. The indications are that neither of these two conditions can be met at present.

In summary, it is hoped that the results of this investigation provide a better understanding of the epizootiology and pathogenesis of ovine coenurosis. It is evident that farm dogs and to a lesser degree hounds are the principal sources of cestode infection, especially T. multiceps in sheep and cattle. Reduction of these infections will result only from regular anthelmintic treatment and ensuring that dogs and hounds do not consume uncooked carcase meat, heads and offal. Some optimism that such practices will be adopted is justified by the fact that ten farmers who regularly reported to the author up to 1987, did so and the incidence of coenurosis was at first reduced and then eradicated from their flocks. Three Hunt Association packs adopted a more strict feeding and anthelmintic treatment regime and the MFH's now claim that the hounds are now virtually cestode free.

The contribution to knowledge of the epizootiology of canine cestodes and ovine coenurosis in Dyfed could, with the benefit of hindsight and in the light of recent reports by other authors, have been enhanced if greater emphasis had been given to some aspects and rather less to others.

Purging of dogs of varying ages on the sheep breeding and wintering farms on at least three occasions over a 12 month period would have demonstrated whether or not

prevalence of infection varied with season, an important factor in the transmission of the parasites between definitive and intermediate host. A similar examination of the hound packs should also have been undertaken for the same reason. In the view of the reports of chronic and acute coenurosis in sheep outside the age range examined in the farm survey, outbreaks of clinical disease, especially in older sheep, should have been investigated in depth, so that the precise age of affected sheep and the circumstances under which such outbreaks occurred could be determined.

The studies on the immunological aspects of T. multiceps metacestode infection should have been modified and extended or abandoned. The extent to which species of cestodes share common antigens was not fully realised, thus reducing the value of the intradermal and complement fixation tests (Chapter 6.3). Subsequent work by the authors quoted in Chapter 6.3 confirm this and clearly indicate the unreliability of this approach. It has been demonstrated that a number of cestode species produce lymphocyte mitogens, T. multiceps being amongst the most potent (Judson, Dixon and Skerrit, 1987). The effect of T. multiceps mitogens on the lympho-reticular system has been studied in detail in vitro (Rakha, Dixon, Skerrit, Carter, Jenkins and Marshall-Clarke, 1991) but no in vivo

studies have yet been carried out. Any progress on the development of a diagnostic test would be dependent on such studies.

Areas which require further research effort include a detailed morphological, and ultimately molecular, of Taenia multiceps and T. serialis work along the lines proposed by Edwards & Herbert (1981) and by Dr M Burt outlined in Chapter 2.1. This, together with experimental infections of a variety of final and intermediate host, would settle the confusion about taxonomic status.

Additional information should be collected to confirm that the deliberate feeding of sheep heads to dogs and hounds is the main or even sole means of transmission. The comparative monitoring of flocks over a period of at least five years on farms where dogs are untreated with those with regular treatment with taenicides would determine the efficacy of treatment as compared to control by the feeding and management of farm dogs.

My thesis has demonstrated feasibility of eradication of ovine coenurosis in spite of the high prevalence of T. multiceps in farm dogs and hounds. The development and availability of effective taenicides could eliminate all species of cestodes from dogs and hounds by regular treatment. However, to prevent re-infection, they should

not be allowed to consume uncooked sheep heads. Reports from the 10 farmers quoted earlier in this chapter indicate that flock eradication is possible, when flocks are self contained and possess mature rams only. However, infection can be introduced through the purchase of ewe and ram lambs and through the overwintering of lambs on dairy farms with T. multiceps infected dogs. Thus complete eradication from a large area or nationally can only be achieved if all farmers implement the full preventive measures described above. As demonstrated in earlier chapters, there is a reluctance to treat dogs regularly and sheep heads were fed on many farms mainly for short term economic reasons. To achieve whole area or national eradication in the absence of a government sponsored scheme, it would be necessary to convince the farming community of the economic benefit of animal health through an extensive publicity and educational programme.

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10 APPENDICES

10.1 Covering letter and questionnaire

GID OR BENDRO IN SHEEP

Since the Veterinary Investigation Centre in Carmarthen opened in September 1969, many farmers, veterinary surgeons and officials of both farming unions, have expressed to me, strong views on the occurrence of gid in sheep throughout the whole of Dyfed.

With the eradication of Brucellosis proceeding rapidly we shall be able to devote more resources to other problems. In view of the concern expressed by so many people about gid we have decided as a first step to collect general information from as many farmers as possible over the next two or three months. I would be grateful therefore if you would kindly complete the attached questionnaire and return it to me as soon as possible and not later than 30 April next. If you have any information which is not specifically asked for, or if you have any other views about the disease could you please pass it on by using the back of the questionnaire.

The information you provide will enable us to plan further work, which we hope will lead to better control and perhaps eradication of the disease from many flocks and areas.

Thank you for your cooperation.

Yours sincerely,

B.M. WILLIAMS

GID FARM SURVEY

REFERENCE _____

Name _____

Address _____

Flock Details:

Self Contained _____ or Flying _____

No of Ewes _____ No of Lambs _____

Have you ever had gid (bendro) diagnosed in your flocks? YES/NO

For how many years have cases been occurring, 1, 2, 3 or more? _____

How many cases per year? _____

In what age groups? Lambs _____

Yearlings _____

More than
1 Yr old _____

How many sheepdogs do you keep? _____

What ages are they? _____

Do you dose them for tapeworms? _____

If so how often are they dosed? _____

Are foxes a problem on the farm? _____

Do you feed your dogs raw meat from

a) sheep you kill on the farm _____

b) carcasses of dead sheep _____

Do you dispose sheep carcasses by

a) burial _____

b)' sending to knackers _____

c) sending to hunt kennels _____

Do you find that sheep carcasses have been attacked by dogs/birds etc before
you find them _____

10.2 List of parishes in the former county of Carmarthen
covered by the questionnaire survey

Cilycwm

Conwil Elfed

Llanddellsant

Llanfair-at-y-bryn

Llangeler

Llangynin

Llansawel

Llanwrda

Meidrim

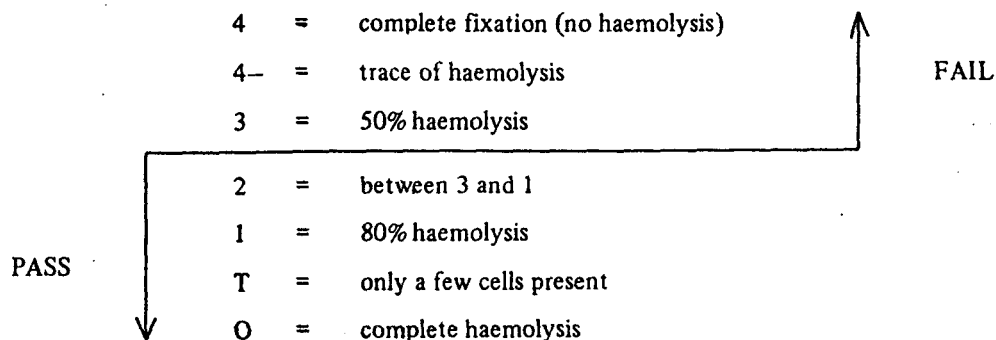
10.3 Details of complément fixation test (from Manual of
Veterinary Investigations Laboratory Techniques Part
2 RUG 3)

COMPLEMENT FIXATION TEST FOR ACID-FAST BACILLI

This test is devised to measure antibodies to acid-fast bacilli in cattle sera. One volume of serum, diluted and heat-inactivated, is incubated at 37°C with one volume of antigen (prepared from a laboratory strain of *M. johnei*) at its titrated optimal dilution and one volume of complement, titrated to contain one 100 per cent haemolytic unit (the highest dilution of complement causing 100 per cent haemolysis of sensitised red blood cells under test conditions).

Antibodies in serum which combine with the antigen will fix some or all of the complement, depending on the antibody concentration, and so less or no complement will be available to lyse the 'haemolytic system' added later. This consists of 3 per cent washed sheep red blood cells sensitised by 5U horse antiserum to red blood cells. The amount of complement in a tube (one unit) is just sufficient to lyse all the sensitised red cells added, so, if some of the complement has already become fixed by antigen-antibody complexes, the tube will contain intact red cells. If sufficient antibody is present no haemolysis will occur at all. After adding the sensitised red cells, the tests are incubated at 37°C for a further 15 min, then left on the bench for 30 min before reading. Appropriate serum, antigen and complement controls are included with each batch of tests.

Results are recorded as follows:



Any serum showing complete fixation at a dilution of 1 in 10 may be taken to titre and the result expressed, for example, as 3/80 i.e. 50 per cent haemolysis at a dilution of 1 in 80.

Reagents

CF buffer diluent. Formula for 2 litres:

| | |
|--------------------|---------|
| Sodium chloride | 85 g |
| Barbituric acid | 5.75 g |
| Sodium barbiturate | 3.75 g |
| Magnesium sulphate | 2.036 g |
| Calcium chloride | 0.294 g |
| Distilled water | 2 l |

Wadsworth, A.B. Standard Methods, 3rd Edition. Bailliere, London.

Method.

1. Add sodium chloride to 500 ml distilled water.
2. Dissolve barbituric acid in 500 ml boiling water and add to CF diluent.
3. Dissolve sodium barbiturate in 500 ml water and add to saline.
4. Dissolve calcium chloride and magnesium sulphate in 500 ml water.
5. Heat and stir.
6. Add this to the rest of the solution.
7. Store at +4°C.
8. Dilute 1 in 5 for use.

Antigen. Antigen will be issued by CVL and should be stored at 4°C. Sufficient antigen for daily use should be heated at 60°C for 30 min before use.

Complement. Commercially available freeze dried.

Haemolytic system

1. Sheep red blood cells (RBC) purchased from Difco Laboratories, in Alsevers solution, and stored at 4-6°C. On the day of use the RBCs are washed with CF buffer, i.e. 5 ml RBC + 5 ml buffer is centrifuged at 2,500 r.p.m. for five min, the supernatant discarded and the packed cells resuspended in buffer. This is done until the supernatant is colourless (usually 2-3 times). The cells are spun down once more and the packed cells resuspended to give a 3 per cent suspension, i.e. 0.6 ml packed RBCs in 20 ml of CF buffer.
2. Horse haemolytic serum is very stable at 4-6°C. One unit or minimum haemolytic dose of serum is the dilution which completely sensitises the red cells to lysis in the presence of excess complement under test conditions. Accurate titration is difficult but horse haemolytic serum from Burroughs Wellcome has invariably given a titre of

one unit equal to a dilution of 1/1,000. Five units are used in the haemolytic system to ensure complete sensitisation of the RBCs, i.e. a dilution of 1/200, e.g. 0.1 ml in 20 ml CF diluent.

3. Equal volumes of 3 per cent washed RBCs and 1/200 horse haemolytic serum are mixed together for 15 min in a 37°C water bath before they are needed, to allow adequate sensitisation of the red cells. Amount of haemolytic system required = number of tubes in test x 0.4 ml.

Glassware

1. Chemically clean glassware must be used, which should be washed thoroughly in tap water followed by several rinses in distilled water. Occasional degreasing and cleaning of tubes may be carried out using 10 per cent HCl, followed by thorough washing as above.

2. Automatic syringes, e.g. Hamilton* syringes may be used to dispense repeated 0.2 ml volumes of the same reagent. They must be rinsed well in CF buffer before use and when changing reagents. Tuberculin syringes are used to dispense sera and other reagents in small volumes and rinsed as above in CF buffer.

3. Measuring cylinders are used to measure large volumes.

Complement titration

1. Dilute antigen 1 in 10 (1 ml antigen stock + 9 ml CF buffer) and place it in a 60°C waterbath for 30 min to inactivate any anti-complementary activity.

2. If freeze dried complement is used carefully follow suppliers directions for reconstituting.

OR

Make complement dilutions in 10 ml beakers. First add 1 ml complement (C) to 9 ml buffer to make a 1/10 dilution. Use this for the following test dilutions (Table 34).

Table 34

| Dilution | 1/20 | 1/30 | 1/40 | 1/50 | 1/60 | 1/70 | 1/80 | 1/90 | 1/100 |
|-----------|------|------|------|--------|--------|--------|--------|--------|--------|
| C' 1/10 | 1 ml | 1 ml | 1 ml | 0.5 ml | 0.5 ml | 0.5 ml | 0.5 ml | 0.5 ml | 0.5 ml |
| CF buffer | 1 ml | 2 ml | 3 ml | 2 ml | 2.5 ml | 3 ml | 3.5 ml | 4 ml | 4.5 ml |

3. Place four rows of nine tubes in a rack. The first two rows will receive one unit volume of a C' dilution and two unit volumes of buffer, and will test the activity of the complement in duplicate. The other two rows will receive one unit volume of C' dilution, one unit volume of antigen and one unit volume of buffer, and will test any lessening or increasing of the complements activity by the antigen.

4. Put 0.2 ml of each complement dilution into one tube from each row, (i.e. four tubes). Add 0.4 ml CF buffer to each tube in the first two rows, and 0.2 ml buffer to each tube in the third and fourth rows. Dilute the inactivated antigen to its appropriate titre, then add 0.2 ml to each tube in the third and fourth rows.

5. Shake all tubes well and incubate for two h in 37°C waterbath to simulate the time allowed for complement fixation in the diagnostic test.

6. Add 0.4 ml sensitised RBCs to each tube. Shake well and return to waterbath for 15 min, then remove them, shake again, and leave undisturbed on the bench for 30 min.

7. Read the titration. The highest dilution of C' causing complete haemolysis of cells will be one unit of complement.

Antigen titration.

1. Make 1 in 10 dilutions of known Johne's positive serum (0.5 ml serum + 4.5 ml CF buffer) and Johne's antigen (0.5 ml antigen stock + 4.5 ml CF buffer) and inactivate them in 60°C waterbath for 30 min.

2. Prepare master dilutions of:

a. Inactivated 1/10 serum. Two fold serial dilutions in five tubes.

CF buffer

1/20 2 ml + 2 ml 1/10 serum: mix well and carry 2 ml to next tube

1/40 2 ml + 2 ml 1/20 serum: " " " " " " " "

1/80 2 ml + 2 ml 1/40 serum: " " " " " " " "

1/160 2 ml + 2 ml 1/80 serum: " " " " " " " "

1/320 2 ml + 2 ml 1/160 serum: mix well and discard 2 ml

b. Inactivated 1/10 antigen (Table 35)

Table 35

| Dilution | 1/30 | 1/60 | 1/90 | 1/120 | 1/200 | 1/250 |
|--------------|------|------|--------|--------|---------|---------|
| 1/10 antigen | 2 ml | 1 ml | 0.5 ml | 0.5 ml | 0.25 ml | 0.25 ml |
| CF Buffer | 4 ml | 5 ml | 4 ml | 5.5 ml | 4.75 ml | 6.0 ml |

3. a. Set up a checkerboard titration in 55 tubes, as illustrated in Table 36. Add 0.2 ml of each serum dilution to each tube of the appropriate row (i.e. horizontal).

Table 36

| Dilution of known positive serum | Dilution of antigen | | | | | | Serum controls |
|----------------------------------|---------------------|------|------|------|-------|-------|---------------------------|
| | 1/10 | 1/30 | 1/60 | 1/90 | 1/120 | 1/200 | |
| 1/10 | 4 | 4 | 4 | 4 | 4 | 1 | 0 |
| 1/20 | 4 | 4 | 4 | 4 | 3 | 0 | 0 |
| 1/40 | 4 | 4 | 4 | 4 | 1 | 0 | 0 |
| 1/80 | 4 | 4 | 4 | 4 | 0 | 0 | 0 |
| 1/160 | 3 | 2 | 1 | 1 | 0 | 0 | 0 |
| 1/320 | 2 | tr | 0 | 0 | 0 | 0 | 0 |
| Antigen controls | 2 | tr | 0 | 0 | 0 | 0 | Sensitised cell control 0 |
| Complement controls | 1.25u | 1.0u | 1.0u | 0.5u | | | |
| (no antigen or serum) | 0 | 0 | 0 | 3 | 3 | | |

b. Add 0.2 ml of each antigen dilution to each tube of the appropriate column (i.e. vertical).

c. Add 0.2 ml of CF buffer to the control row and column.

d. Add 0.2 ml of complement (one unit as previously titrated) to each tube, and set up five tubes with complement controls as in Table 37:

Table 37

| Units of C' | 1.25 | 1 | 1 | 0.5 | 0.5 |
|-------------|---------|--------|--------|--------|--------|
| Complement | 0.25 ml | 0.2 ml | 0.2 ml | 0.1 ml | 0.1 ml |
| CF buffer | 0.35 ml | 0.4 ml | 0.4 ml | 0.5 ml | 0.5 ml |

4. Shake all tubes well and incubate in 37° waterbath for two h.
5. Add 0.4 ml sensitised RBCs to each tube, shake well and return to waterbath for 15 min. Remove them, shake well and leave undisturbed on bench for 30 min before reading.
6. In the above example of checkerboard antigen titration the optimal dilution of antigen to be used is 1/90, i.e. the highest dilution of antigen which gives maximum fixation of complement (and therefore no haemolysis) and has no anti-complementary activity. The antigen dilutions of 1/10 and 1/30 show anti-complementary activity, seen in the antigen control row, but the serum control column reveals no anti-complementary activity. The complement controls show that complement was used at the optimal dilution, since half a unit of complement gives 50 per cent haemolysis and 1 unit gives 100 per cent haemolysis.

Diagnostic complement fixation test

Screening blood samples

1. Spin blood samples in centrifuge at 2,500 r.p.m. for five min, then dilute each serum 1 in 10 (0.1 ml serum + 0.9 ml CF buffer). Dilute a known Johne's positive serum 1 in 10 and inactivate with test sera at 60°C for 30 min.
2. Dilute sufficient antigen for the day's tests, 1 in 10. (Amount of final dilution of antigen required = 0.2 ml x no. of sera to be tested + 2.2 ml for controls).
3. Set up two tubes per serum sample and add 0.2 ml of the diluted inactivated serum to each. One tube tests the serum for the presence of antibodies to the antigen used, and the other is a serum control to observe any anti-complementary activity in the serum.
4. *Positive control.* Make six serial dilutions of the inactivated 1/10 positive serum unless a titre is known, to check that the test procedure gives its correct titre (Table 38).

Table 38

| Dilution | 1/10 J+ve serum | CF buffer | Procedure |
|----------|-----------------|-----------|---|
| 1/10 | 0.2 ml | — | |
| 1/20 | 0.2 ml | 0.2 ml | Mix well and transfer 0.2 ml to next tube |
| 1/40 | — | 0.2 ml | " " " " " " " " |
| 1/80 | — | 0.2 ml | " " " " " " " " |
| 1/160 | — | 0.2 ml | " " " " " " " " |
| 1/320 | — | 0.2 ml | Mix well and discard 0.2 ml |

Also put 0.2 ml J+ve serum into an anticomplementary tube.

5. Dilute the inactivated 1/10 antigen to its previously determined optimal titre. Add 0.2 ml antigen to all tubes except anticomplementary controls and complement controls. Add 0.2 ml buffer to anticomplementary controls.
6. Dilute sufficient frozen complement (freshly thawed) to give the correct titre (1 unit in 0.2 ml). Amount of final dilution of C' required = 0.4 ml x no. of test sera + 3.1 ml for controls. Add 0.2 ml C' to all tubes except complement and antigen controls.

7. *Complement controls*, to test that C' is being used at correct dilution (Table 39).

Table 39

| Units of C' | 1.25u | 1 u | 1u | 0.5u | 0.5u |
|-------------|---------|--------|--------|--------|--------|
| CF Buffer | 0.35 ml | 0.4 ml | 0.4 ml | 0.5 ml | 0.5 ml |
| C' 1 unit | 0.25 ml | 0.2 ml | 0.2 ml | 0.1 ml | 0.1 ml |

- Antigen controls*, to test that antigen is not affecting the activity of the complement (Table 40).

Table 40

| | | | | | |
|-----------|---------|--------|--------|--------|--------|
| CF Buffer | 0.15 ml | 0.2 ml | 0.2 ml | 0.3 ml | 0.3 ml |
| C' 1 unit | 0.25 ml | 0.2 ml | 0.2 ml | 0.1 ml | 0.1 ml |
| Antigen | 0.2 ml | 0.2 ml | 0.2 ml | 0.2 ml | 0.2 ml |

8. Shake all tubes well and incubate in 37°C waterbath for two h, 15 min before the tubes are due out, have haemolytic system ready.
9. Remove all tubes, add 0.4 ml sensitised RBCs to each one. Shake well and replace in 37°C waterbath for 15 min. Remove and leave undisturbed on bench for a further 30 min before reading.
10. *Reading.* Make a 50 per cent haemolysis tube by mixing 0.5 ml of a completely haemolysed sample (e.g. the 1.25u complement control) with 0.5 ml buffer. Estimate degree of haemolysis in the supernatant of each tube. If cells have not settled, spin them down before reading. Any serum showing complete fixation at a dilution of 1 in 10 may be titrated out, twofold serial dilutions (see 'Positive serum control').